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Journal of the Fisheries Research Board of Canada

Experimental Approaches to Limnology — An Overview

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OUR early attempts to study the Experimental Lakes were based largely upon standard observational limnological methods (see J. Fish. Res. Board Can. 28(2)). Once we began studying the response of lakes to fertilization, it quickly became obvious that such methods were giving us both superficial and erroneous glimpses of the dynamics of a lake's response to nutrient enrichment. The papers in this volume are the product of 4 years of searching for methods to describe and interpret changes in lakes of the Experimental Lakes Area, as well as related laboratory work which illuminates certain critical problems in freshwater nutrient dynamics. This paper will attempt to summarize these contributions.

Dissolved Inorganic Carbon (DIC)

One of the greatest shortcomings we encountered was the lack of a reliable method for measuring DIC in ELA lakes. These data are extremely important since the oligotrophic nature of lakes in the area made measurement of phytoplankton production by the oxygen bottle technique impractical, and in order to use the ^{14}C method the DIC concentration must be known. Unfortunately, concentrations of DIC were so low, and buffering by humic compounds so high, that the standard titration method of determining HCO_3^- (for example, American Public Health Association 1965), then calculating DIC from HCO_3^-, *p*H, and temperature proved too inaccurate to be useful. Suitable techniques employing concentration of DIC by various chemical means were extremely slow and required large samples, so that only a limited number of analyses could be performed per day. This problem is not one unique to ELA; it is common throughout the Canadian Shield in areas with little calcareous drift or where acid fallout has reduced DIC concentrations (Schindler and Nighswander 1970; Armstrong and Schindler 1971; Johnson and Michalski 1970). Large areas of Scandinavia are similar. Standard techniques for DIC determination were either unsuitable or barely adequate for 50% or more of the world's fresh waters!

The first part of this issue of the Journal of the Fisheries Research Board of Canada is made up of papers on "Experimental Limnology." Because the 13 papers are so interrelated, reprints of individual articles are not available. Requests for additional copies of these papers should be addressed to the *Librarian, Department of the Environment, Fisheries and Marine Service, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6.*

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Since production estimates by direct measurement of DIC were desired, a method which would work with small samples was necessary, eliminating currently available infrared techniques.

The modified gas-chromatographic (GC) technique of Stainton (1973) solves the above problems. It offers the convenience of rapid, accurate analysis on small samples, for a wide range of DIC concentrations. Because a syringe serves both as a sampling device and equilibration chamber, it offers a number of advantages for production or microstratification studies (Schindler et al. 1973a). Moreover, DIC, the measurement required for most primary production studies, is measured directly, not calculated from bicarbonate, temperature, and hydrogen ion concentration. Bicarbonate and other forms of DIC if desired for chemical calculations, can be back-calculated by applying correction for *p*H and temperature (Park 1969). The technique is, unfortunately, rather expensive and unsuited for work in remote areas.

Other problems with calculation of DIC from titration results may be encountered in more alkaline lakes and oceans. For example, corrections for ion pairing have been a major source of controversy in such calculations (Wangersky 1972). When making corrections for ionic activity it is usually assumed that all important elements dissolved in lake water are in true ionic solution. ELA staff (unpublished data) have recently found that a high proportion of these elements may be connected to colloidal material (the same fraction mentioned by Lean (1973a, b) in connection with phosphorus). If this problem is widespread, it could seriously complicate the calculation of ion-pairing effects. The GC technique should therefore prove invaluable in water bodies of all types.

Adenosine Triphosphate (ATP) Measurements

A second difficult problem we encountered was the lack of a simple method for measuring live microplankton biomass. Algal counts and volume calculations are tedious and rather imprecise, and it is often impossible to separate living organisms from dead, but well-preserved ones. Chlorophyll measurements are difficult to interpret, because standard corrections for degradation products do not appear to be reliable. The equipment and time required for reliable corrections are beyond the scope of routine studies (Daley et al. 1973). Moreover, it is possible for viable undegraded chlorophyll to be preserved for long periods after cells are functionally dead, particularly in cold, anoxic hypolimnions or in sediments (Vallentyne 1955).

Measurement of ATP has been done in marine systems for several years (Holm-Hansen and Booth 1966). In fresh waters it may offer a number of advantages over algal counts and chlorophyll measurements. It is extremely sensitive, and amenable to use on a routine basis (Rudd and Hamilton 1973). The compound degrades quickly once cells die, so that it should provide a reliable estimate of live microplankton. There are, however, a number of possible problems which must be thoroughly tested. Since ATP is a component of all living organisms, it does not allow phytoplankton to be separated from bacteria or zooplankton. In some waters size separation can be accomplished to some degree by screening samples through different mesh sizes, but results are still difficult to interpret. Also, it is still not known how good an estimate of phytoplankton activity ATP may provide, since no author has compared ATP content with photosynthetic activity. Further work is required.

Phosphate

One of the major remaining analytical chemistry problems is the estimation of true phosphate concentrations. Standard acid molybdate methods give results which are far too high in most waters (Rigler 1966; ELA staff unpublished data). Bioassay or sephadex techniques (Rigler 1968; Lean 1973a, b) allow maximum estimates of PO_4 to be made: they are, however, time-consuming, and require highly experienced personnel. Additional serious problems with analytical chemistry are discussed below.

Physical Method – Diffusion Gas Exchange

Physical methods useful for experimental purposes were found to be as wanting as biological or chemical ones. Although we observed soon after fertilizing lake 227 that an external source of carbon was needed to account for the observed increase in algal biomass, we had no way of knowing whether this came from atmosphere, sediments, or hypolimnion. Atmospheric invasion of gases has not previously been measured in freshwater lakes although there are a few crude estimates in the literature. The direct CO_2 technique (Schindler and Fee 1973) and the radon technique (Emerson et al. 1973) allow at least order of magnitude estimates to be made, and additional refinement appears possible. The uptake of radium by littoral diatoms complicated measurements of gas exchange by the radon method (Emerson and Hesslein 1973). These problems have been overcome in more recent experiments (S. Emerson unpublished data).

It was necessary to measure vertical and horizontal eddy diffusion coefficients to evaluate the contribution of DIC and other nutrients from the hypolimnion and

littoral sediments. Once again, standard techniques were not suitable. Dye diffusion provides a short-term idea of movement. However, to obtain any idea of what is happening over long time periods repeated injections of dye must be made. The classical heat-flux method for measuring vertical eddy diffusion is not sensitive enough to allow short-term resolution of data. It also assumes that diffusion of heat and molecular diffusion occur at identical rates. Problems are discussed by Hutchinson (1957).

The radon technique for measuring diffusion (Hesslein and Quay 1973) is not encumbered by these problems. The radon generator (RAGAPOS) can be inserted into the lake in the desired position, then left for a few days until disturbances associated with the insertion have disappeared. Afterward, monitoring of radon concentrations above and below the RAGAPOS at any time allows short-term, i.e. several days, estimates of vertical eddy diffusion throughout the year, once radioactive decay of the radon is corrected for. The generator requires only occasional replenishment with radium 226. A slight modification of the apparatus also allows horizontal eddy diffusion to be estimated (Hesslein and Quay unpublished data). The method has one great disadvantage when compared to the other two methods — radon measurements are more difficult and time-consuming than dye or temperature measurements.

Biological Methods

Traditional in situ methods for measuring primary production (for example Vollenweider 1969, Schindler and Holmgren 1971) were found to be inadequate for our studies. Schindler and Fee (1973) clearly demonstrated the inadequacy of the ^{14}C bottle techniques in low DIC situations like lake 227. Similar problems would be expected in other eutrophic lakes with low DIC, and in those subjected to acid fallout.

Because of the unpredictable nature of natural incident light, comparison of primary production between lakes or dates when results are based on in situ data, is nearly impossible to do in a valid manner. Moreover, with standard in situ methods, synoptic data for a variety of stations or lakes cannot be easily obtained. The incubator technique and numerical methods of Fee (1973a, b) offer solutions to these problems. Fee's (1973b) methods for calculating production accommodate irregular distribution of surface light, extinction of light in water, and phytoplankton distribution. This approach shows considerable promise in environmental studies, since it does not demand the rigid fitting of mathematically complex but biologically obscure functions in order to incorporate data into predictive models. Such techniques have far more promise for modelling ecological systems than traditional, mathematically oriented engineering methods. They offer the nearest thing to an "ecological mathematics" which is likely to develop for some time. Schindler et al. (1973a) have demonstrated that in addition to the above-mentioned light problem, substrate heterogeneity and methodological inconsistencies severely hamper the interpretation of periphyton production measurements. Additional study, along the lines used by Fee in the above-mentioned phytoplankton studies, appears to be in order.

Our results reveal that the study of algal nutrient limitation needs rethought, much careful experimentation, examination, and redirection. Not only is it likely that there are different limitations to both rate of production and eventual standing crop (Schindler and Fee 1973), but as for any stress, both the duration and degree of the limitation must be accounted for. The study of "limiting concentrations" may need to be replaced with a more dynamic concept. Diurnal and seasonal aspects, as well as the lake's history, must be taken into account. It is extremely difficult to determine from nutrient bioassay studies which nutrient was originally limiting to algal populations in a modified lake. Such bioassays must be conducted before, as well as after the modification to allow sound interpretations to be made. Techniques for making such measurements in a valid manner, with the natural algal flora of the lake, must be developed.

Production as it is usually measured by the ^{14}C technique is really a measurement of the flux of one nutrient, carbon. It is strange that the study of biological effects of other nutrients has been largely limited to determination of concentrations, or to comparison of these concentrations to fluxes of carbon. The studies by Lean (1973a, b), Schindler and Fee (1973), and Thompson and Hamilton (1973) demonstrate the necessity of considering fluxes of nutrients among several "pools" in an ecosystem to understand what is happening. The elegant treatments of rate problems by Riggs (1963) and Rigler (1972) should prove useful as models for such studies for a variety of important nutrients. The above-mentioned papers, as well as those employing isotope pairs for gas exchange and diffusion (Emerson et al. 1973; Hesslein and Quay 1973), derive far more information from isotope techniques than is usually done. Additional work, both conceptual and experimental, is required to understand how the interrelationships of cycles of various nutrients affect phytoplankton populations.

Consideration of whole lake balances for various nutrients (Schindler et al. 1973b) together with studies of cycling rates (for example Lean 1973a, b; D. R. S. Lean and D. W. Schindler unpublished data; Peters and Lean 1973) reveals that the usual models of nutrient dynamics disregard some of the most important components of nutrient cycles. As shown in Table 1, internal

TABLE 1. Average loss and recycling of epilimnion total phosphorus and carbon via different pathways, August 1971. All data are as percent of total element per day. Data are from Schindler et al. (1973b), D. R. S. Lean and D. W. Schindler (unpublished data), and R. Peters (unpublished data).

	P	C
Outflow	0.01	0.01
Sedimentation	3.7	2.1
Grazing	8–11	8–11
Excretion	≃ 15,000	≃ 300

cycles of phosphorus, involving uptake of phosphate by phytoplankton and its regeneration by plankton excretion and grazing, are far more rapid than external ones. It is possible that productive research could be directed toward controlling internal cycling mechanisms in attempts to suppress algal blooms. The different relative transfer rates shown for carbon and phosphorus in lake 227 also cast serious doubt on the validity of stoichiometric analyses of nutrient availability in relation to plankton composition. Again, our results merely offer a suggestion, and further investigation is needed.

Conclusion

A number of friends and colleagues have been surprised and disappointed when they have learned that we do not have a "model" or "systems analysis" for lake 227, where so much effort has been spent. The reason for this is a conviction by the primary project scientists (Schindler, Fee) that current approaches to systems modelling are based on erroneous assumptions. For example, if we had made only the traditional production and nutrient measurements on lake 227, then incorporated the usual assumptions that production is linearly related to surface light, that production or algal standing crop is a function of nutrient concentration, and that exchange of DIC with the atmosphere is negligible, it will be obvious to the reader that our model would have told little about actual mechanisms controlling algal production or standing crop in the lake. While it is obvious from the papers by Fee, Lean, and Emerson that we have not ignored modelling entirely, the belief that whole aquatic ecosystems may be productively analyzed without years of painstaking study of mechanisms is largely fictitious. From a purely scientific point of view, the most important area in limnological research remains the construction and thorough testing of a sound methodology, designed to illuminate ecosystem problems rather than just to describe them. I hop that a few preliminary steps in the right direction may be found among the contents of this volume.

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The assistance and enthusiasm of a number of persons made these papers a pleasure, rather than a chore, to produce. K. Patalas, A. L. Hamilton, and J. R. Vallentyne all read several papers and provided helpful criticism and suggestions. E. Marshall located a number of difficult-to-find references. Publication would not have been possible without the patience, care, and efficiency of three people. G. Porth typed several drafts of most of the papers. C. Boyce drafted, and usually redrafted, most of the figures from crude and often inadequate instructions. V. E. Frost assisted with a multitude of corrections, proofreadings, and other editorial chores.

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Eutrophication of Lake 227 by Addition of Phosphate and Nitrate: the Second, Third, and Fourth Years of Enrichment, 1970, 1971, and 1972

D. W. SCHINDLER, H. KLING, R. V. SCHMIDT, J. PROKOPOWICH, V. E. FROST, R. A. REID, AND M. CAPEL

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SCHINDLER, D. W., H. KLING, R. V. SCHMIDT, J. PROKOPOWICH, V. E. FROST, R. A. REID, AND M. CAPEL. 1973. Eutrophication of lake 227 by addition of phosphate and nitrate: the second, third, and fourth years of enrichment, 1970, 1971, and 1972. J. Fish. Res. Board Can. 30: 1415–1440.

Lake 227, a small lake with extremely low concentrations of dissolved inorganic carbon, was fertilized with PO_4 and NO_3 for 4 years, beginning in 1969. The additions increased natural inputs of phosphorus and nitrogen about five times.

Phytoplankton standing crop increased nearly two orders of magnitude, and the Cryptophyceae and Chrysophyceae present in natural lakes of the area were replaced by Chlorophyta and Cyanophyta. The standing crop of phytoplankton per square meter was near the maximum which could theoretically be maintained by surface light, in spite of the low carbon concentrations. Added phosphate and nitrate were rapidly removed by phytoplankton, so that concentrations in the lake remained low.

Almost all of the added nutrient was retained by the lake, in spite of relatively fast water renewal times. An average of 80% of the phosphorus income of the lake was sedimented. There was no return of phosphorus from sediments in spite of anoxic conditions in the hypolimnion.

Photosynthesizing plankton reduced dissolved inorganic carbon concentrations severely, causing a flux of atmospheric CO_2 into the lake. From 69 to 95% of the inorganic + particulate carbon supplied to the lake was from the atmosphere. Results demonstrate that low carbon concentrations do not hinder eutrophication if phosphorus and nitrogen supplies are adequate.

SCHINDLER, D. W., H. KLING, R. V. SCHMIDT, J. PROKOPOWICH, V. E. FROST, R. A. REID, AND M. CAPEL. 1973. Eutrophication of lake 227 by addition of phosphate and nitrate: the second, third, and fourth years of enrichment, 1970, 1971, and 1972. J. Fish. Res. Board Can. 30: 1415–1440.

Le lac 227, un petit lac à concentrations excessivement faibles de carbone inorganique dissous, a été fertilisé au PO_4 et NO_3 pendant 4 ans, à commencer en 1969. Ces additions ont augmenté d'environ cinq fois l'apport naturel de phosphore et d'azote.

La biomasse du phytoplancton a presque doublé, et les Chlorophyta et Cyanophyta ont remplacé les Cryptophyceae et les Chrysophysaceae présents dans les lacs naturels de la région. La biomasse du phytoplancton par mètre carré a presque atteint le maximum que peut théoriquement maintenir la lumière de surface, en dépit de faibles concentrations de carbone. Le phosphate et le nitrate ajoutés ont été rapidement absorbés par le phytoplancton, de sorte que les concentrations dans le lac sont demeurées faibles.

Presque toutes les substances nutritives ajoutées ont été retenues par le lac, en dépit de taux de renouvellement de l'eau relativement rapides. Environ 80% en moyenne des entrées de phosphore du lac ont été absorbées par les sédiments. Il n'y a pas eu libération de phosphore par les sédiments, en dépit des conditions anoxiques de l'hypolimnion.

La photosynthèse par le plancton a sérieusement abaissé les concentrations de carbone inorganique dissous, causant un flux de CO_2 atmosphérique dans le lac. De 69 à 95% du carbone inorganique + particulaire reçu par le lac provenait de l'atmosphère. Ces résultats démontrent que des basses concentrations de carbone n'empêchent pas l'eutrophisation, si les apports de phosphore et d'azote sont suffisants.

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AN earlier paper (Schindler et al. 1971) reported results of the first year of fertilization with nitrate and phosphate of lake 227, a small lake in the Canadian Shield. This paper reports results of the second, third, and fourth years of the fertilization program, 1970, 1971, and 1972, and further calculations based on more complete data.

Methods

Methods used and measurements made in 1970, 1971, and 1972 were unchanged from those in 1969 with the following exceptions: phosphoric acid (H_3PO_4) was substituted for the Na_2HPO_4 used as a phosphate sou ce in 1969, because of the greater solubility of the former. Total additions of nu rients were 0.48 g P/m^2 and 6.29 g N/m^2/year, slightly higher than additions in 1969. The above amounts of nutrients were divided into 21 equal portions, which were added weekly between mid-May and mid-October. The lake was sampled every week during the ice-free seasons of 1969, 1970, and 1971, biweekly in 1972, and roughly monthly when ice-covered, at depths of 0, $\frac{1}{2}$, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 m.

Methane and dissolved organic carbon in lake 227 were measured only in 1971 and 1972, using gas chromatography (Stainton 1973; ELA staff unpublished data).

Soluble reactive phosphorus measurements were not made in 1972, for reasons discussed later.

The mass of nitrogen, phosphorus, carbon, and other elements in the water column was calculated on several dates in 1970 and 1971, by using morphometric data for the lake (Brunskill and Schindler 1971) and concentrations of each element at each depth (C_z) including both suspended and dissolved fractions. The mass of the element in the lake (M) on any date was calculated from the formula:

$$M = \int_{z_o}^{z_m} C_z A_z \, dz \qquad (1)$$

where A_z is the area of a contour at depth z, and z_m = maximum depth of the lake. The equation was solved for 11 dates in each year for each element, except that only nine dates were possible for nitrogen in 1970, due to the absence of total nitrogen data in June and July, 1970.

Sedimentation[1] (S) of nitrogen or phosphorus during a period was calculated by subtracting changes in mass of an element in the lake during each time interval ($\triangle$M) and effluent from the lake (E) from input to the lake (I):

$$S = I - \triangle M - E \qquad (2)$$

[1]Sedimentation as used here for the elements C, N, and P refers to movement to the bottom of the lake by any gravitational means, including sinking of phytoplankton and chemical precipitation.

Calculations were done for periods of approximately 4 weeks in summer, while the entire winter (under ice cover) was considered to be one period.

Input of an element to the lake was considered to be the sum of amounts entering the lake with precipitation, runoff, and added fertilizer:

$$I = P + R + F \qquad (3)$$

Amount of precipitation and its nutrient content were assumed to be the same as in the nearby basin of lake 239, where all precipitation falls were measured and analyzed. Volume and concentration of nutrients in runoff were calculated using data from the northwest subbasin of lake 239 (ELA staff, unpublished data). Measurements on a variety of ELA watersheds indicate that the error due to this assumption should be less than 50%, which is insignificant when natural inputs are compared to fertilizer additions.

Equation (3) as applied to nitrogen ignores nitrogen fixation and denitrification. Preliminary measurements by Flett (1972) using the acetylene method indicate that summer fixation of nitrogen in lake 227 is nearly as high as fertilizer additions. Further work is in progress (R. J. Flett personal communication), and will be treated at a later date.

Equation (1) was also used to compute the mass of C, N, and P at the end of the period of winter stratification and at the end of the period of summer stratification, in 1969 (where data was sufficient), 1970, 1971, and 1972. The purpose of this analysis was to determine whether there were measurable year-to-year increases in the elements in the water column of the lake.

Preliminary gas-exchange experiments (Schindler and Fee 1971) indicated that a substantial portion of the carbon input to the lake was via net invasion of CO_2 from the atmosphere (A_C). This amount should be equal to:

$$A_C = S_C + \triangle M_C + E_C - I_C \qquad (4)$$

Where S, $\triangle$M, E, and I are as defined above and the subscript C indicates carbon. S_C for each budget period was calculated on the basis of the average C to P ratio in suspended (and therefore sedimenting) matter. The average suspended C:P ratio in the lake was used for this calculation. Tests for analytical and sampling error indicated that the above calculations could be done with precision sufficient for the interpretations made here (R. Wagemann and M. Stainton unpublished data).

Changes in Physical Factors

Summer temperatures were quite similar in 1969 and 1970, with epilimnion temperatures of 20–23 C throughout June, July, and August (Fig. 1; Schindler 1971, fig. 6f). In contrast, 1971 and 1972 epilimnetic temperatures averaged about 2 C lower in late July and early August due to cold, stormy weather (Fig. 1).

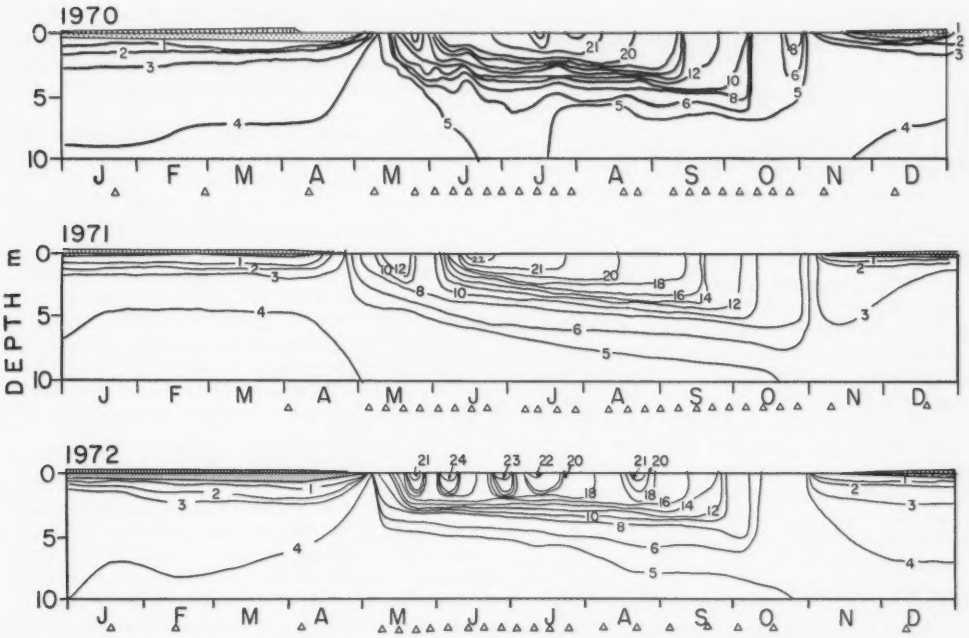

FIG. 1. Seasonal temperature isopleths, C, lake 227, 1970–72. △ on this and following figures designate sampling dates. Barred and strippled areas represent snow and ice, respectively.

Secchi disc visibility was as low as 0.4 m in July of 1972, compared with values of 2.2–2.5 m prior to fertilization (Fig. 2). Values in 1971 were low early in the season, but high in late July and early August, during a period of cold weather and a zooplankton maximum.

Phytoplankton Standing Crops and Species Composition

Only a few pre-fertilization phytoplankton data are available for lake 227 (Schindler et al. 1971). Due to the more detailed information on similar, unfertilized lakes (the Class B lakes of Schindler and Holmgren 1971 and Kling and Holmgren 1972) which has been collected in the past several years, it is now possible to infer more about the lake prior to fertilization.

Winter live biomass averages about 1000 mg/m^3 in such lakes. Summer maxima seldom exceed 4000–5000 mg/m^3, and average ice-free season values would be about 2000 mg/m^3. Corresponding chlorophyll-*a* concentrations are 0.5–2 µg/liter in winter and 1–5 µg/liter during the ice-free season.

Chrysophyceae dominate these small Shield lakes all year. *Chrysochromulina parva* var., *Chromulina* spp., *Chrysococcus* spp., *Kepherion* spp., *Pseudokepherion* spp., and *Botryococcus braunii* are common in winter. In spring *Dinobryon* spp., *Chrysochromulina parva* var., *Mallomonas pumilio* var., *Chrysoikos skujai*, *Pseudokepherion* spp., and *Kepherion* spp. are dominant. Summer species are nearly unchanged from spring ones. In fall a mixture

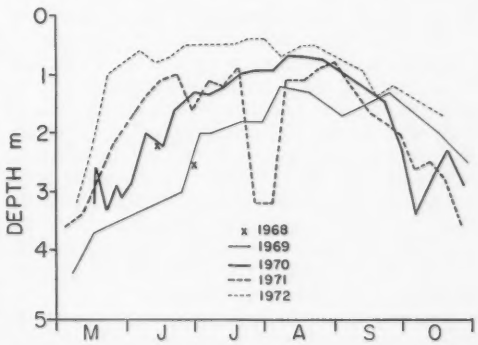

FIG. 2. Seasonal changes in Secchi disc visibility, lake 227, 1968–72.

of summer and winter forms prevails. Kling and Holmgren (1972) give further description.

Post-Fertilization Changes

In 1969, the first year of fertilization, species were primarily Chlorophyta in summer, reaching standing crops as high as 50 µg/liter chlorophyll, or 16160 mg/m^3 live biomass. Winter chlorophyll and live biomass were nearly identical to values for unfertilized lakes (Schindler et al. 1971).

In 1970 blue-green algae, which were relatively scarce in 1969, the first year of fertilization, absent in 1968, and rarely detected in the epilimnions of unfertilized lakes, became a late summer dominant in 1970 (Fig. 3). Winter species of phytoplankton, present until mid-May, were similar to those described for the winter of 1968–69 (Schindler et al. 1971; Kling and Holmgren 1972). By late June, 1970, the dominant species had changed to *Staurastrum paradoxum*, *Staurastrum dejectum*, *Staurastrum boreale*, *Sphaerozosma granulatum*, *Spondylosium planum*, *Chroococcus limneticus*, *Mallomonas elongatum*, *M. caudata*, *M.* cf. *intermedia*, *M. pumilio* var. *canadensis*, and *M. pseudocoronata*. This domination by Chlorophyta was very similar to that observed in late summer of 1969. In July Cyanophyta began to increase in importance, reaching a maximum abundance in mid-September. Most important species were *Oscillatoria geminata*, *O.* cf. *amphigranulata*, and *Pseudoanabaena articulata* in July and August, joined by *Lyngbya lauterbornii* in mid-August. Other species present in reasonably large numbers at the time were a colorless *Gymnodinium* sp., a number of *Trachelomonas* spp., *Pseudomallomonas* sp., the protozoans *Coleps hirtus*, and *Gymnodinium* cf. *varians*, in late July and early August; and *Difflugia* spp. and other zooflagellates, plus *Spondylosium planum* in late August and early September. Bacillariophyceae never formed a significant component of the phytoplankton standing crop in 1970 (Fig. 3). In late September and October chrysophycean and cryptophycean genera common during the previous winter (Schindler et al. 1971) reappeared even though fertilization continued until the end of October, indicating that temperature or light rather than nutrient supply was the factor primarily responsible for return to winter species composition. Chrysophyceans appeared much earlier in the fall in 1970 than in 1969, probably because of cooler weather earlier in the fall in the latter year.

Phytoplankton standing crop in 1970 was nearly double that of 1969, with late summer chlorophyll-*a* concentrations, uncorrected for phaeophytins or other degradation products, reaching 90 µg/liter (Fig. 4; Schindler et al. 1971, Fig. 6 and 7) and live cell volumes in excess of 35,000 mm^3/m^3. The 1970 maximum standing crop was also reached earlier, in late August, compared to mid-September in 1969.

The 1971 winter and spring phytoplankton was dominated by the same chrysophycean and cryptophycean species as in the previous 2 years (Fig. 3). The biomass did not increase greatly until June, when epilimnion temperatures approached 20 C. At that time the *Oscillatoria* spp. which were common in the fall of 1970 reappeared in large numbers. These were displaced for a short period in mid-June, when a rapid pulse of diatoms occurred, dominated by *Synedra acus* var. *radians* and *Synedra nana*. Diatoms decreased rapidly after silicon was depleted (Fig. 11b), and filamentous bluegreens (chiefly *Oscillatoria*) were again dominant by the end of the month.

In July and early August, during a period of unseasonably cool weather when epilimnion temperatures dropped to 18 C from June values of 22–23 C (Fig. 1), Chlorophyta began to increase in importance. Dominant species were different from earlier years, with *Dictyosphaerium elegans*, *Oocystis submarina* var. *variabilis*, *Ankistrodesmus falcatus* var. *spirale*, *Chlorella* sp., *Scenedesmus* spp. *Gleotilopsis* sp., and *Chlamydomonas* spp. most abundant. By mid-July they exceeded Cyanophyta in abundance. Chrysophycean species typical of the winter plankton (*Mallomonas* spp., *Chromulina erkensis*, *Dinobryon* spp.) reappeared about the same time.

In early August a tremendous decline occurred in all phytoplankton groups, simultaneous with sharp increases in Rotifera and Protozoa. Recovery began by mid-August, when the euglenophytes *Trachelomonas* spp., *Euglena* spp., *Astasia* sp., and *Lepocinclis* sp. increased. By late August Chlorophyta were again dominant, with *Spondylosium planum*, *Oocystis submarina*, and *Scenedesmus* spp. of major importance.

Typical winter chrysophycean and cryptophycean genera returned in late September and October (*Botryococcus*, *Ochromonas*, *Mallomonas*, *Dinobryon*, *Chrysococcus*, *Chromulina*, *Cryptomonas*, and *Rhodomonas*).

Epilimnetic cell volume during the late summer of 1971 was much lower than in 1970, with a maximum of 12,000 mm^3/m^3 (Fig. 3b), probably as a result of cold temperatures, low light, and a massive rotifer population during that period. On the other hand, the chlorophyll-*a* maximum in the metalimnion and upper hypolimnion (up to 296 µg/liter) in September 1971 (Fig. 4b), was the highest concentration ever recorded in lake 227 or any other

lake in the area. Production at this depth was extremely low and the concentration of adenosine triphosphate (ATP) negligible (Rudd and Hamilton 1973) so we assume that the chlorophyll was detrital, probably resulting from the accumulation of sinking dead phytoplankton.

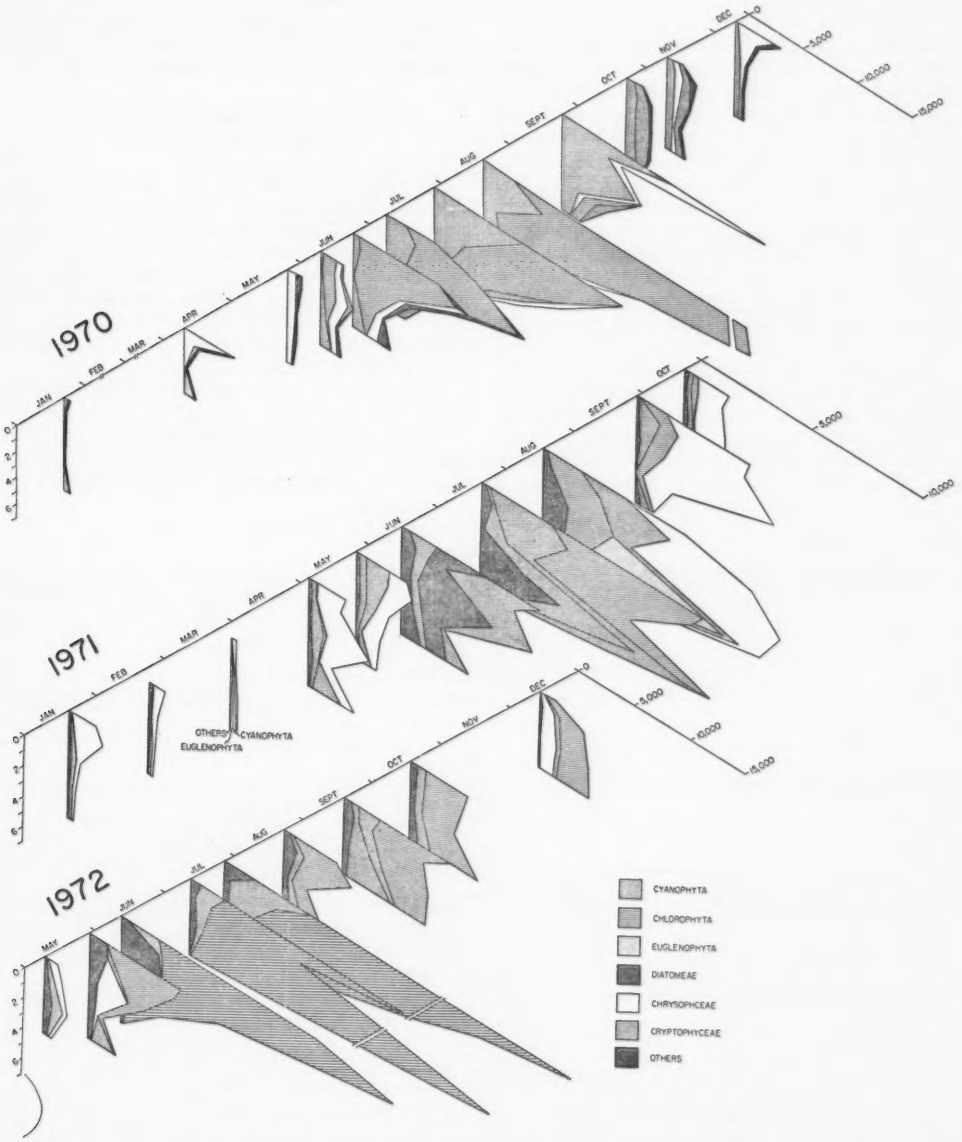

FIG. 3. Seasonal changes in abundance and depth-distribution of major phytoplankton groups, lake 227, 1970–72. Units are mm^3/m^3.

The 1972 winter (January–April) species were the same as in previous years (Fig. 3c). In mid-May, after ice left the lake, *Oocystis submarina* var. *variabilis*, *Scenedesmus* spp., *Dictyosphaerium elegans*, *Ankistrodesmus falcatus* var. *spirale*, and other Chlorophyta began to increase in the epilimnion, while the cyanophytes *Oscillatoria redekia* and *Oscillatoria* sp., became important in the hypolimnion. These species moved upward into the epilimnion in June and July, but were not able to exceed the above-mentioned Chlorophyta, which continued to dominate the phytoplankton throughout the summer and autumn. The maximum reached was 63,300 mg/m^3 on July 27, followed by a decline in biomass throughout August, September, and October. Chrysophyceae and Crytophyceae, which had returned by mid-autumn in other years, did not return by the end of December, and the total phytoplankton biomass at that time was higher than in earlier years.

Euglenophyta and Bacillariophyceae did not form a significant proportion of the biomass at any time in 1972. A complete list of phytoplankton species and abundance is on file at the Freshwater Institute.

Phytoplankton production has proved extremely difficult to measure due to low concentrations of dissolved inorganic carbon. Nearly all production in midsummer takes place in the early morning hours, when phytoplankton deplete the CO_2 regenerated by respiration and invasion during the night. Preliminary measurements in several area lakes indicate that phytoplankton production in lake 227 may be about 10 times higher than in natural lakes of the area (Schindler and Fee 1971).

Changes in Nutrient Concentration in the Water Column

No study published to date has documented chemical changes in an experimentally-fertilized lake for several years after fertilization was begun. Such changes have been the subject of much speculation in the recent controversy over limiting nutrients, and poorly supported assumptions have become major cornerstones in the argument. It therefore appears desirable to give all chemical data, particularly for P, N, and C.

As in 1969, the major response to fertilization in 1970, 1971, and 1972 occurred in the suspended (sestonic) fractions (Fig. 5; Schindler et al. 1971, Fig. 10, 11, and 16). Once again, increases in seston-C in the epilimnion were observed which were

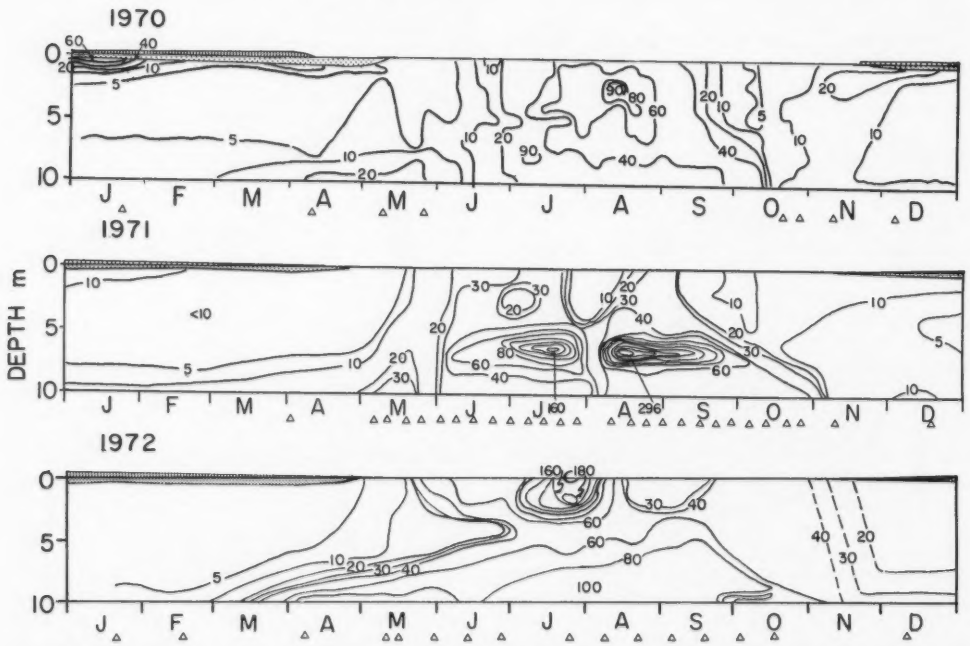

FIG. 4. Seasonal isopleths for chlorophyll-*a*, μg/liter (uncorrected for phaeophytins), lake 227, 1970–72.

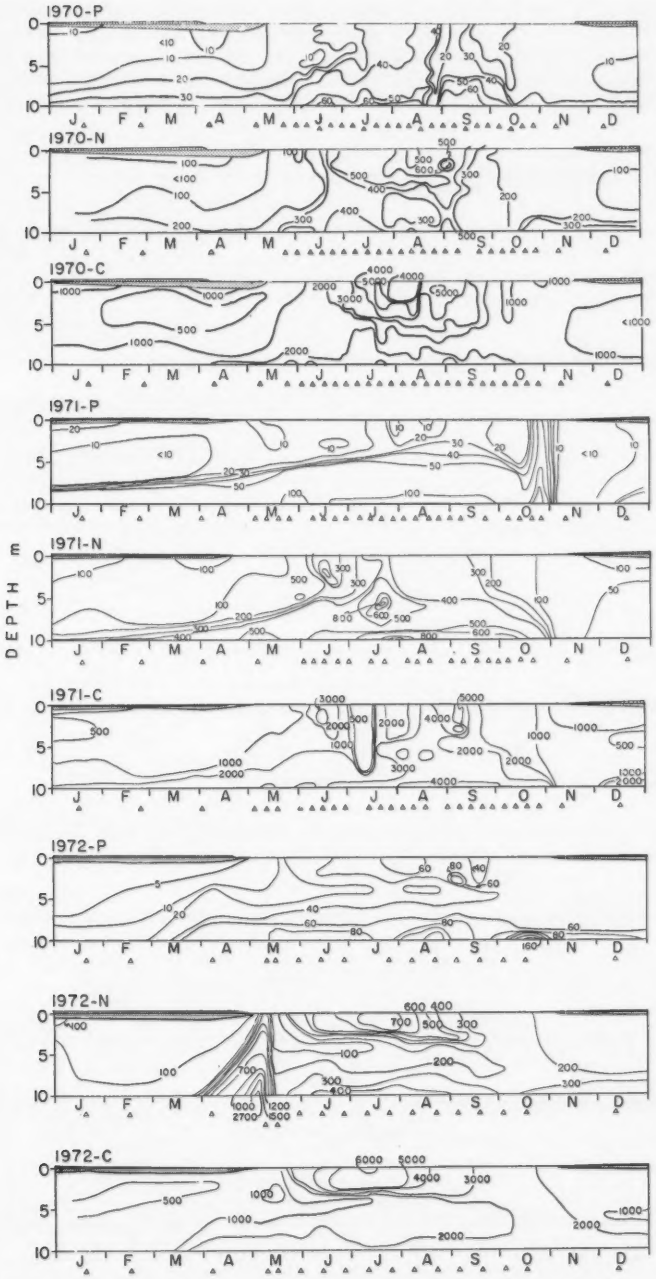

FIG. 5. Seasonal isopleths of suspended particulate (sestonic) phosphorus, nitrogen and carbon, μg/liter, 1970–72.

equivalent to those for P and N (Fig. 5c, f, and i), even though no carbon was added with fertilization. The increase in sestonic carbon did not cause corresponding decreases in epilimnetic concentrations of dissolved inorganic carbon fractions, indicating that these were replenished from some outside source. The maximum epilimnetic concentrations observed for all elements were in July 1972. Cold temperatures, low light, and enormous rotifer populations in late July of 1971 and August of 1972

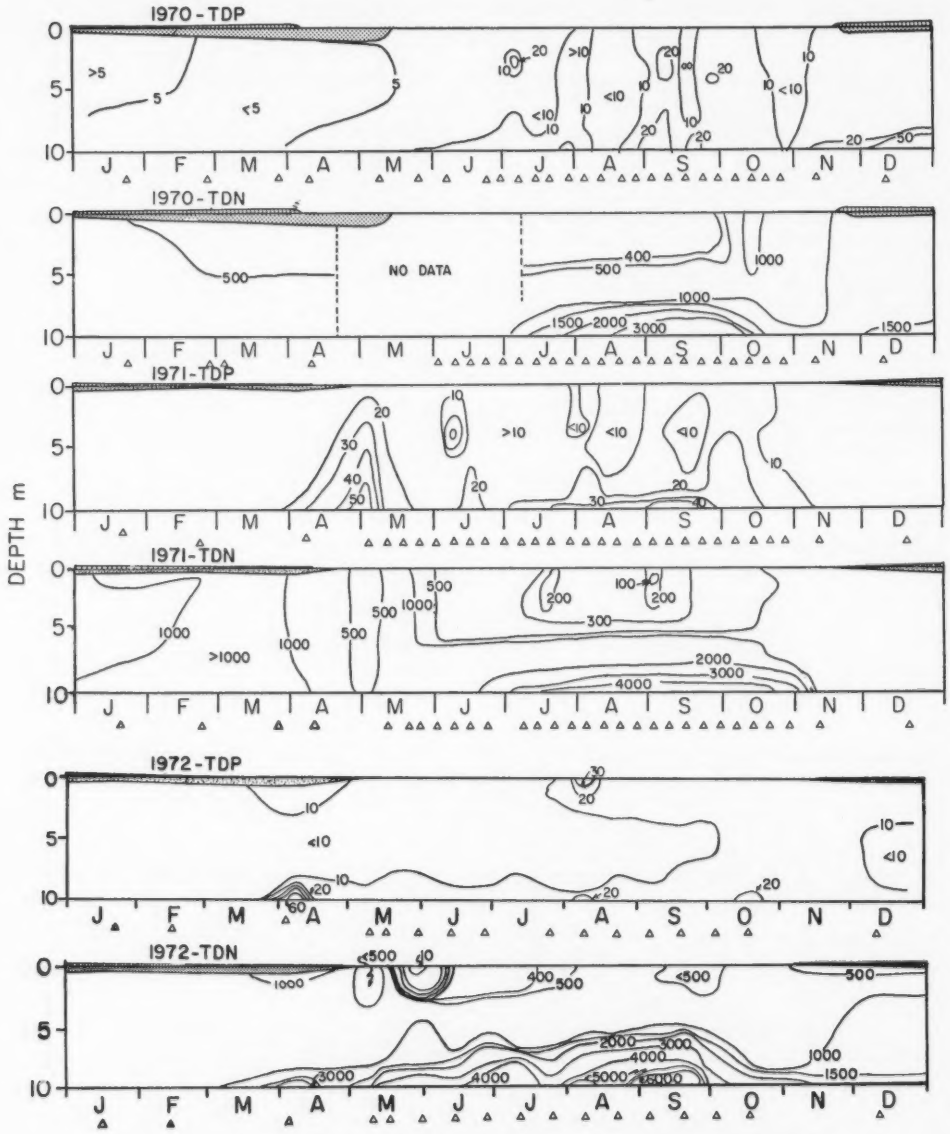

FIG. 6. Seasonal isopleths of total dissolved phosphorus and nitrogen, μg/liter, 1970–72.

probably caused the smaller epilimnetic algal populations and sestonic C, N, and P concentrations during those periods.

Little or no increase was noticed in epilimnetic concentrations of total dissolved nitrogen and phosphorus during the 4 years of fertilization (Fig. 6; Schindler et al. 1971, Fig. 9 and 13). Hypolimnetic concentrations of TDN did increase, due chiefly to large quantities of ammonia produced by decaying plankton (Fig. 6b, d, and f; Schindler et al. 1971,

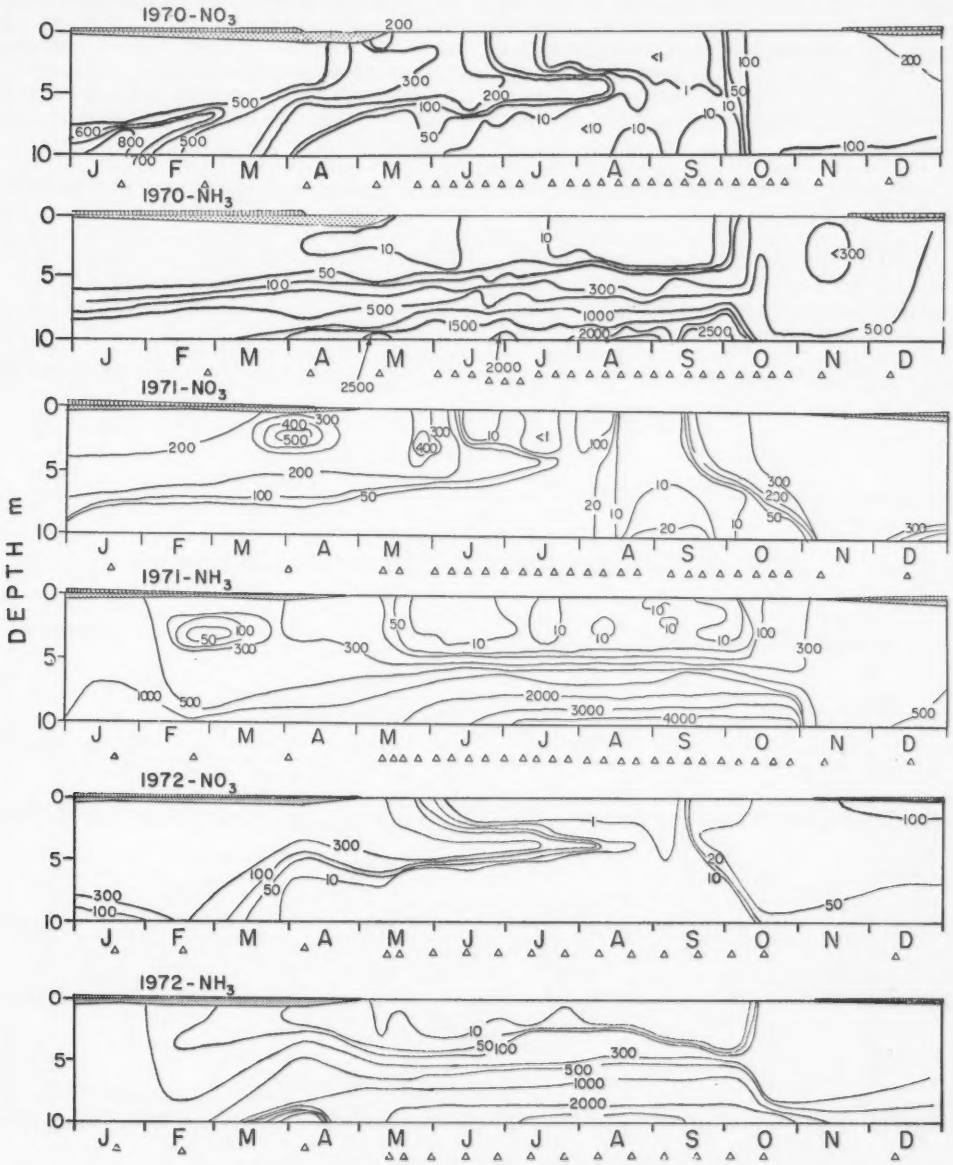

FIG. 7. Seasonal isopleths of dissolved inorganic nitrogen, μg/liter, 1970–72.

Fig. 14). Although several methods have been tested, we have yet to find a completely reliable means of analyzing high concentrations of total dissolved nitrogen in the hypolimnions of Canadian Shield lakes. Since most of the dissolved nitrogen in the hypolimnion appeared to be ammonia (Fig. 7b, d, and f), the error is probably not great, and results should be considered as slight underestimates.

Nitrate, nitrite, and molybdate-reactive phosphorus concentrations remained low in both epilimnion and hypolimnion (Fig. 7 and 8). Soluble reactive phosphorus values (Fig. 8; Schindler et al. 1971, Fig. 8) indicate that the added phosphate (approximately 10 µg P/liter of epilimnion per week) disappeared rapidly and continuously, since values were usually below the limits of detectability for the method (1 µg/liter). Even these are overestimates of the true concentration of PO_4-P in the lake, since the molybdate complex reacted with phosphorus hydrolyzed from colloidal organic compounds under acid conditions (ELA staff, unpublished data). Measurements with a bioassay

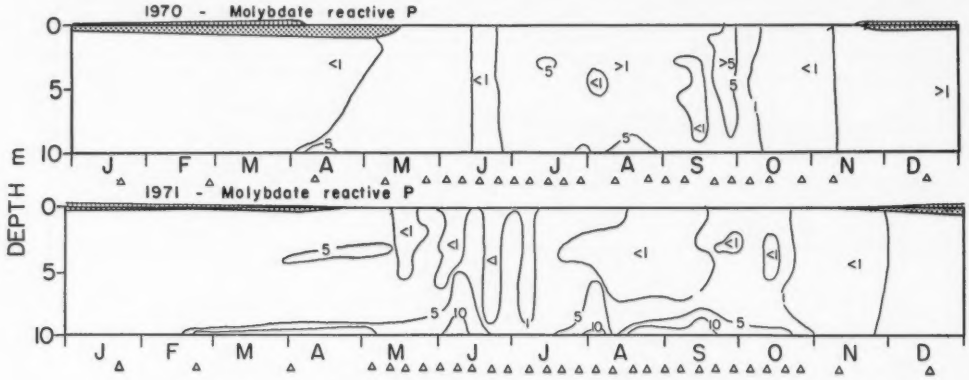

FIG. 8. Seasonal isopleths of soluble reactive phosphorus, µg/liter, 1970–71.

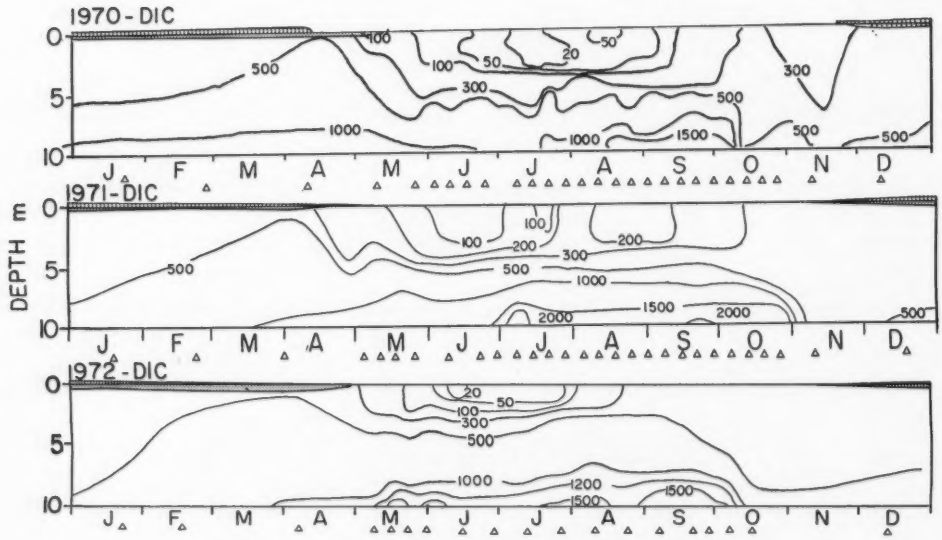

FIG. 9. Seasonal isopleths of DIC, µmoles C/liter, 1970–72.

technique (Rigler 1966) revealed that concentrations of true PO_4-P in the epilimnion of lake 227 seldom reached 0.05 µg/liter during the late summer, fall, and winter of 1971 or spring of 1972 (D. W. Schindler unpublished data). Because of the above problem the standard acid molybdate test was dropped from ELA analyses at the end of 1971.

Epilimnetic dissolved inorganic carbon (DIC) in 1971 remained at much higher concentrations than in 1969, 1970, and most of 1972 (Fig. 9; Schindler et al. 1971, Fig. 15). A similar period of high epilimnetic CO_2 occurred after the rapid decline of a large phytoplankton standing crop in August of 1972. There was a progressive increase in dissolved inorganic carbon throughout the 4 years of fertilization, due to invasion of atmospheric CO_2 (Schindler et al. 1972). This is discussed in more detail later. The greater epilimnetic DIC in 1971 probably also reflects the smaller epilimnetic standing crop of phytoplankton, and therefore lower demand for carbon, than in 1969 and 1970.

There was relatively little seasonal change in dissolved organic carbon (DOC) during 1971 and 1972 (Fig. 10a, c) Epilimnetic concentrations of 600–800 µmoles/liter were common throughout the summer of 1971, with slightly lower values (300–500 µmoles/liter) in 1972. Concentrations in the deeper hypolimnion reached 1000–2000 µmoles/liter in late summer probably due chiefly to decay of sedimenting plankton. There was no accumulation of DOC above the sediments during winter, which one would expect if the material were of sedimentary origin. There is little temperature difference between summer and winter at the sediment surface, so the difference in DOC is probably due to higher sedimentation in summer rather than higher rates of microbial action.

Methane was over 1000 µmoles/liter at the mud–water interface in July of 1971 when analyses were begun (Fig. 10b, d). It doubled during the next 2 months, then disappeared nearly entirely at fall overturn. Accumulation began again under winter ice, at a rate somewhat slower than in summer.

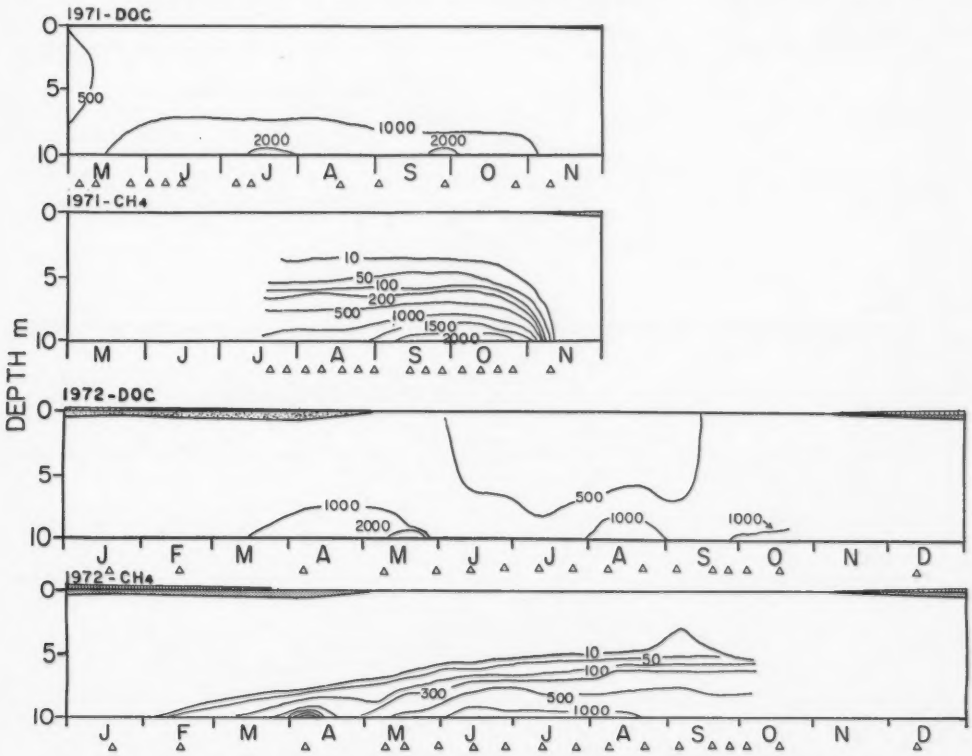

FIG. 10. Seasonal isopleths of dissolved organic carbon, µmoles/liter, 1971 and 1972.

Concentrations just above the sediments were lower in 1972, but there was an increase in total mass of CH_4-C in the lake over 1971.

Changes in Concentrations of Other Elements

The June depletion in epilimnetic silica observed in 1971 was much greater than in 1969, 1970, and 1972 (Fig. 11; Schindler et al. 1971, Fig. 22), probably because of the greater abundance of diatoms in that year.

The seasonal pattern of oxygen concentration was very similar in the summers of 1969, 1970, 1971, and 1972 (for example Schindler et al. 1971, Fig. 2). This is to be expected, since epilimnetic concentrations are determined largely by atmospheric saturation and temperature, while the hypolimnion of the lake was anoxic even prior to fertilization. Seasonal isopleths for all years are therefore not shown, but year-to-year changes will be discussed later.

No significant change in seasonal distribution patterns of Ca, Mg, or K has been noted over the 4 years, indicating that these elements are not limiting to the phytoplankton of lake 227. Isopleths are not shown.

Sodium concentrations in the epilimnion increased continuously through the summer season each year, due to the addition of $NaNO_3$. Little of the sodium reached the hypolimnion while the lakes stratified, (Fig. 12; Schindler et al. 1971, fig. 18) indicating that algal demand for the element was low in relation to supply, and that the added fertilizer was effectively confined to the epilimnion during summer stratification.

Hydrogen ion concentration changes 3 to 4 orders of magnitude in the epilimnion in summer (Fig. 13 and Schindler et al. 1971, fig. 17). This is highly important because of its relation to partition of DIC into HCO_3^-, $CO_3^=$, and gaseous CO_2 ($CO_{2(aq)}$). At low midsummer concentrations of DIC, a *p*H of 10 has been shown to chemically enhance the

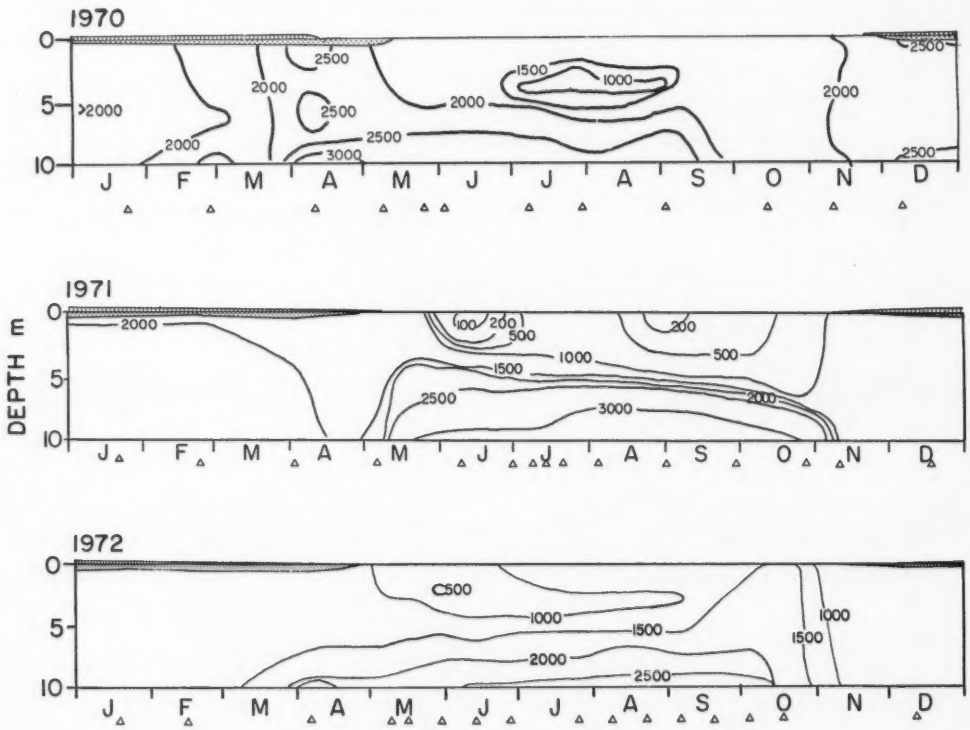

FIG. 11. Seasonal isopleths of reactive silicate, μg Si/liter, 1970–72.

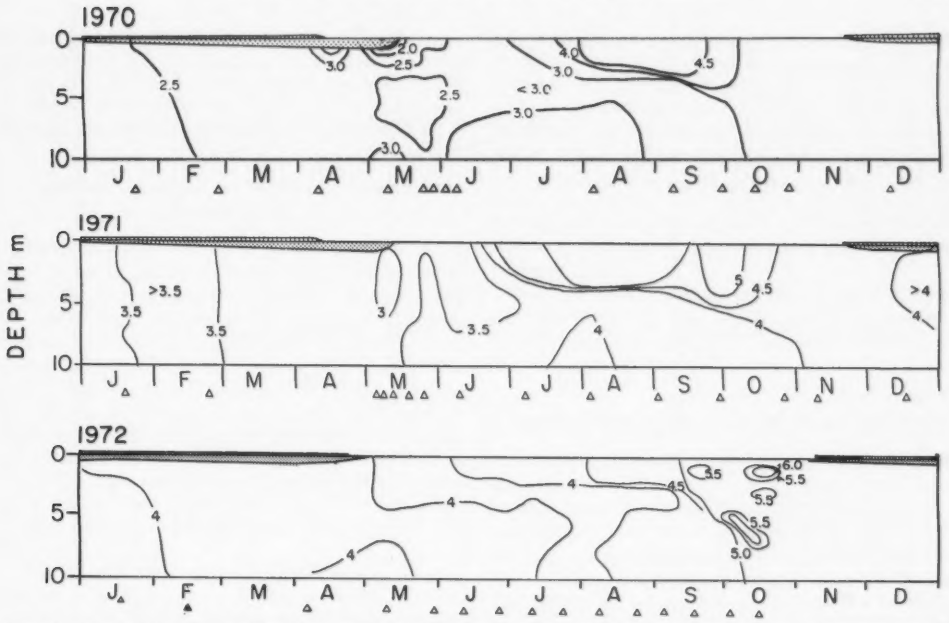

FIG. 12. Seasonal isopleths of sodium, mg/liter, 1970–72. The high epilimnetic concentrations in summer are due o addition of $NaNO_3$ fertilizer. The absence of movement of sodium from epilimnion to hypolimnion during the summer is regarded as an index of low demand by phytoplankton.

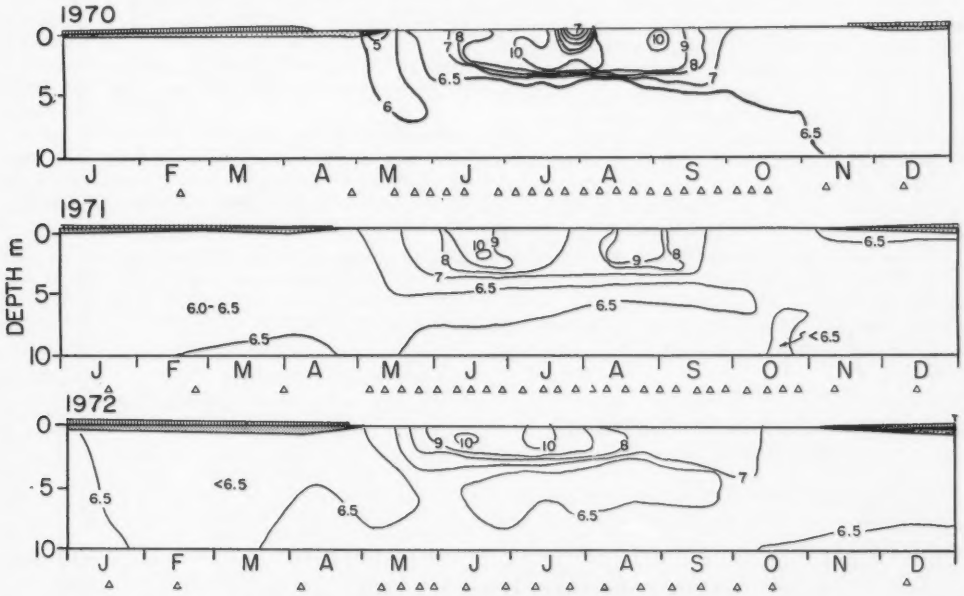

FIG. 13. Seasonal *p*H isopleths, lake 227, 1970–72.

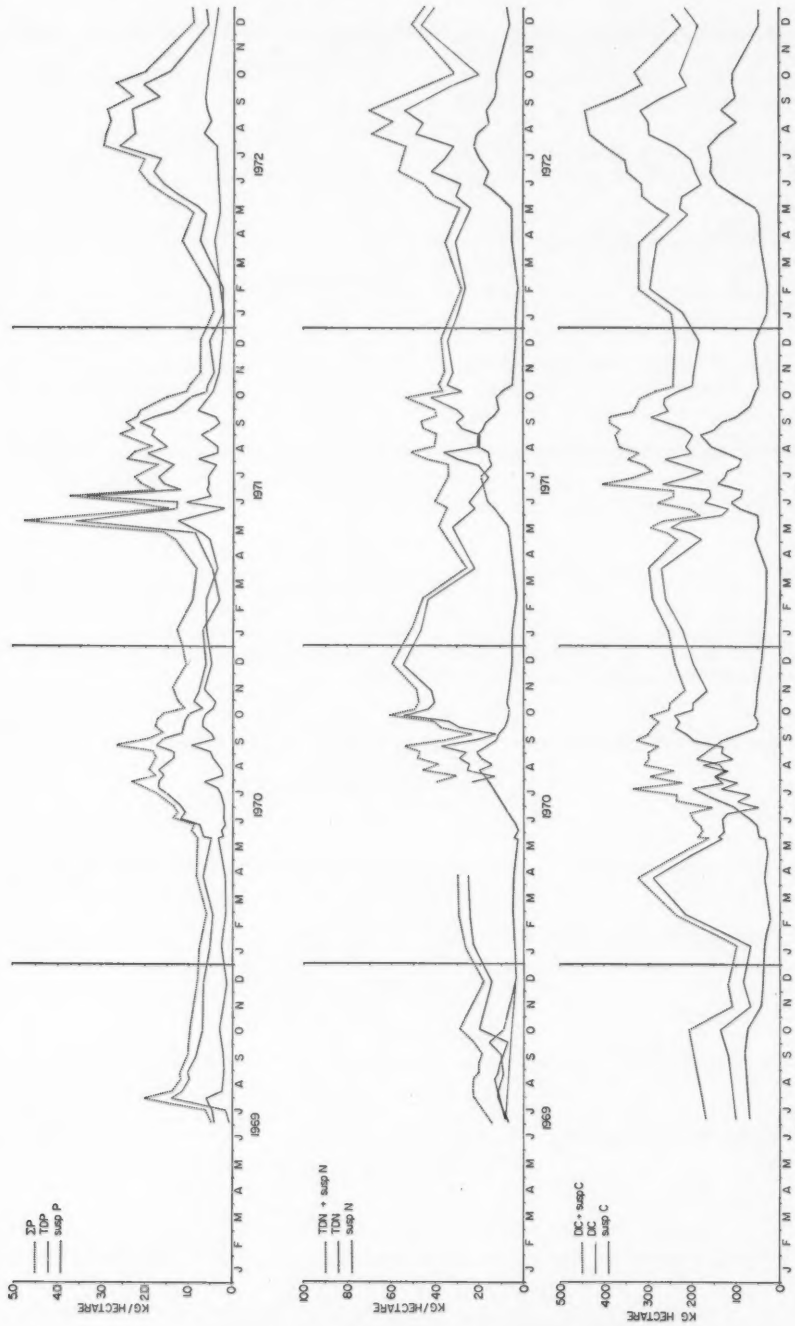

FIG. 14. The mass of dissolved, suspended, and total nutrients in the water column of lake 227 since the start of fertilization in 1969. See text for discussion of seasonal patterns and long-term trends.

invasion of CO_2 from the atmosphere to the epilimnion by 3 to 4× because rapid hydroxylation of the gas to HCO_3^- as it enters the lake keeps its partial pressure in the epilimnion much lower than in the atmosphere (Schindler et al. 1972).

Chronic Changes in Mass of Nutrients in the Lake

Although there was an apparent increase in the total mass of nutrient in the lake each summer (Fig. 14), such results are difficult to interpret due to year-to-year variation in seasonal chemical regimes. To test the significance of results, data were arbitrarily divided into summer (May–October) and winter (November–April). Differences in average masses of element in the whole lake between years were then analyzed by analysis of variance, followed by Duncan's New Multiple Range Test (Li 1957) when significant differences between years were found. "Significant" in the following paragraphs means statistically significant at the 5% level.

The average total mass of phosphorus in summer increased each summer, averaging 1.97 kg/year, or about 7% of the average annual input of the element. The rate of increase decreased with time, with significant increases occurring in 1970 and 1971, but not in 1972 (Table 1).

Average nitrogen also increased each summer, averaging 54.9 kg/year. Increases were statistically significant in 2 years out of 3 (Table 1).

Summer carbon showed large and statistically significant increases from year to year, averaging 258 kg/year (Table 1).

Winter results are in striking contrast to summer ones. For all three nutrients, differences between mean masses in different years were not significantly different. These results suggest that although summer values are continuously increasing, perhaps as the result of sedimentary return or more efficient retention of nutrient in the water column plus (in the case of carbon and perhaps nitrogen) atmospheric gas exchange (see below), the annual winter minimum in all nutrients has not been affected by fertilization. Since the lake is sealed by ice from atmosphere and outflow throughout the winter, and losses of element occur largely in the suspended fraction, sedimentation of plankton must be responsible.

Seasonal cycles of gain and loss of carbon were complex (Fig. 14c), due largely to seasonal patterns of stratification and phytoplankton activity in the lake (see below).

TABLE 1. A summary of results of analysis of variance and multiple range tests on summer (May through October) masses of nutrients, other elements, and chlorophyll-*a* in lake 227. All significance levels are 95%.

Element	Difference between means
P	1969 < 1970 < (1971 = 1972)
N	1969 < (1970 = 1971) < 1972
C	1969 < 1970 < 1971 < 1972
O_2	1969 < (1970 = 1971) < 1972
H^+	(1969 = 1970) < (1971 = 1972)
Si	1969 < (1970 = 1971 = 1972)
Chlorophyll-*a*[a]	(1969 = 1970 = 1971) < 1972

[a]Epilimnion values only.

Chronic Changes in Mass of Other Elements

There has been a year-to-year trend for the minimum amount of oxygen in the lake in late winter to decrease (Fig. 15). Due to the small number of samples each winter, the average winter masses are not, however, significantly different. Suffocation of fishes in the lake is certain to take place within the next winter or two, if the present trend continues.

The average summer oxygen content of the lake has also decreased slightly with time, due largely to the more rapid and complete depletion of oxygen in the upper hypolimnion. This observation is significant at the 5% level in 2 years out of 3.

Average mass of hydrogen ion has also decreased (Fig. 15) with 1971 and 1972 significantly lower than 1969 and 1970 (Table 1). This is undoubtedly due to photosynthetic activity of the plankton, which can be visualized as removing a hydrogen ion for each molecule of CO_2 consumed. As found for other elements, winter H^+ content did not change significantly from year to year. Decomposition of the plankton is undoubtedly responsible for regeneration of H^+ in winter.

Average summer silica in the lake dropped significantly between 1969 and 1970, then showed little change (Fig. 15, Table 1). As observed for other elements that were monitored, average winter amounts did not differ significantly from one year to the next.

Chronic Changes in Phytoplankton Due to Nutrient Addition

Epilimnetic, rather than total pigments in the lake were used to analyze trends in standing crop, since primary production and ATP measurements indicated that most of the phytoplankton in the metalimnion and hypolimnion was dead or inactive (Rudd and Hamilton 1973).

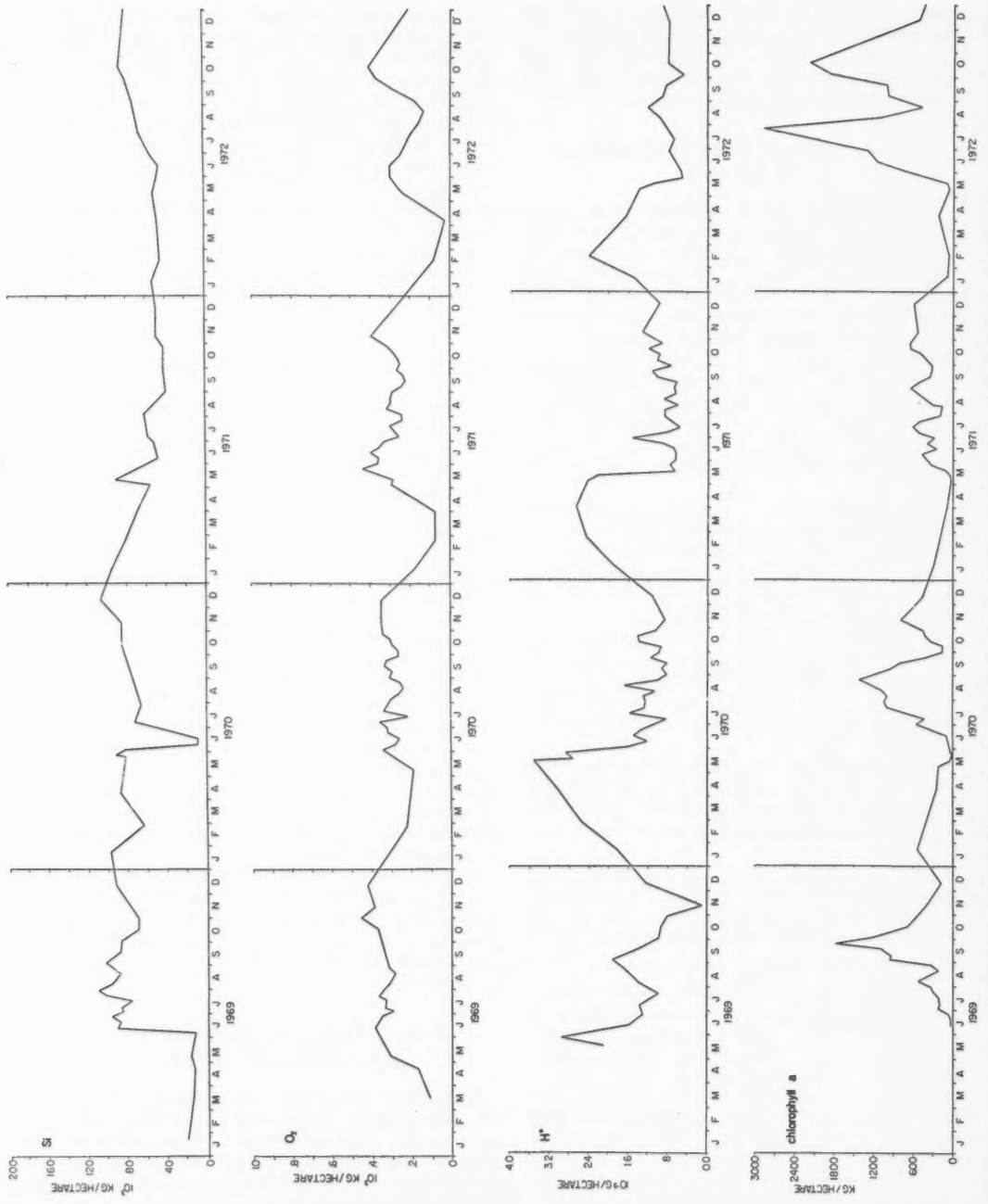
Si
O_2
H°
chlorophyll a
10³ KG/HECTARE
10⁶ G/HECTARE
KG/HECTARE
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1969
1970
1971
1972

FIG. 15. The mass of various chemicals in the water column, 1969–72, lake 227. See text for discussion.

Summer chlorophyll in 1972 was significantly higher than in 1969, 1970, and 1971 (Fig. 15, Table 1), in spite of the fact that fertilizer additions in 1970, 1971, and 1972 were equal. This may be because green algae, rather than bluegreens, dominated the 1972 plankton at the time of maximum standing crop. In 1969, when green algae were dominant, standing crops were as high as 1970 and 1971, even though fertilizer additions were lower in 1969.

Winter chlorophyll content of the lake did not change significantly from 1968–69 through 1971–72. In December of 1972, however, higher values were observed than in other years, possibly due to the abnormally thin snow cover on the ice in November and early December.

Species composition of the winter plankton also changed for the first time in December of 1972. Instead of the usual chrysophycean dominants, the chlorophyceans *Oocytis*, *Ankistrodesmus*, and *Dictyosphaerium*, which had persisted throughout the summer and fall, were still present, as described above. Further observation will be required to tell whether this change is due to chance, or to the continued fertilization of the lake.

Sources and Fates of Added Nutrients

Phosphorus — Input of phosphorus from natural sources was 17–18% of the total, including fertilizer (Table 2). The most concentrated source of this element was rainfall directly on the lake surface, which accounted for about half the natural input in spite of representing only a fraction of the catchment area of the terrestrial drainage.

Nearly all of the incoming phosphorus (84 and 85% in 1970 and 1971, respectively) was retained by the lake. Outflow figures were nearly identical in the 2 years, at 16 and 15% of income.

Of phosphorus retained, nearly all was sedimented, averaging 80% of input. The apparent difference between 1970 and 1971 is due to the fact that the phosphorus content of the water column increased slightly in 1970, then decreased slightly in 1971. Sedimentation averaged 1.2 and 2.6% of the suspended phosphorus in the lake per day for the 2 years. Values were highest in late summer and fall, exceeding 8%/day in 1971, and lowest under winter ice. The negative sedimentation in April and November–December of both years is probably due to suspended materials stirred up from littoral sediments during spring and fall overturn.

Nitrogen — The proportion of incoming nitrogen from natural sources was only slightly higher than that for phosphorus, 19–20% of the total input (Table 3). As for phosphorus, dissolved nitrogen in rainfall directly on the lake surface was the most important natural source.

Retention by the lake was lower and more variable than for phosphorus, at 57 and 71% of input in 1970 and 1971, respectively. In 1970, much of the retained nitrogen remained in the water column, so that sedimentation was only 0.8% of total input. Sedimentation was far more important in 1971, when the amount of nitrogen going to the sediments was only 2% less than total input to the lake. Average sedimentation for the 2 years was 50% of input.

On a seasonal basis, late summer and fall sedimentation rates for nitrogen were highest, reaching values in excess of 4% per day. These are much lower than values for phosphorus, reflecting a decline in N/P ratio with increasing depth. In both years, there was an apparent net increase of nitrogen in the late fall. This may be due to the analytical difficulties connected with high TDN concentration as discussed above, since values from the lower hypolimnion just prior to fall overturn were probably the most serious underestimates of the year. Average daily sedimentation in the 2 years was greatly different, 0.02 and 2.9%, reflecting the average increase in water column values in 1970, and decrease in 1971.

Carbon — Inputs of DIC and suspended carbon from precipitation and runoff were low, representing 5 and 31% of total input in the 2 years. The reason for the much higher value in 1971 cannot be explained with certainty, but since the *p*H of inflows was higher ($\bar{x} = 5.52$, compared to 5.20 in 1970), more DIC may have been lost to the atmosphere in 1970.

Atmospheric gases and sedimentary decomposition contributed most of the carbon entering the lake, 95 and 69% in 1970 and 1971. Most of this was retained, with outflow equal to 18 and 34% of total income from all sources in the 2 years.

Daily sedimentation of carbon was much lower than phosphorus and slightly lower than nitrogen, reflecting the fact that the C/P ratio in suspended matter decreased with increasing depth. Suspended C/P ratios in the epilimnion averaged 111:1 in both 1970 and 1971, while hypolimnetic values averaged only 67:1 and 50:1 in the 2 years.

Figure 14c illustrates the complex seasonal exchange which takes place between atmosphere and lake. During winter, DIC in the water column increases due to decomposition of plankton and washed in forest litter at the mud surface (Fig. 14c, Table 4). Because the lake is sealed by a cover of ice, none of this DIC is lost to the atmosphere,

TABLE 2. A summary of input, output, sedimentation, and changes in the water column

Period 1970	Input (I)							Mass in water	
	Inflows		Precipitation and dustfall		Total natural	Fertilizer	Total	Date	susp P
	susp P	TDP	susp P	TDP					
								Jan. 1	2.90
Jan. 1–Apr. 8	0.00	0.00	0.20	0.49	0.69	0.00	0.69	Apr. 9	3.25
Apr. 9–May 27	0.01	0.44	0.19	0.11	0.75	0.00	0.75	May 23	3.19
May 28–July 1	0.06	0.06	0.16	0.12	0.40	5.70	6.10	June 30	5.81
July 2–July 29	0.00	0.02	0.03	0.14	0.19	5.70	5.99	July 28	8.34
July 30–Sept. 2	0.00	0.01	0.04	0.04	0.09	5.70	5.79	Sept. 1	5.64
Sept. 3–Oct. 7	0.39	0.13	0.43	1.03	1.98	6.84	8.82	Oct. 6	5.27
Oct. 8–Nov. 11	0.23	0.21	0.07	0.12	0.63	0.00	0.63	Nov. 10	3.61
Nov. 12–Dec. 9	0.20	0.02	0.04	0.03	0.29	0.00	0.29	Dec. 9	3.29
Dec. 10–Dec. 31	0.00	0.00	0.02	0.02	0.04	0.00	0.04		
Annual total	0.89	0.89	1.18	2.10	5.06	23.94	29.10		
% of Total input	3.1	3.1	4.1	7.2	17.4	82.3	100.0		
1971									
								Jan. 1	3.35
Jan. 1–Apr. 4	0.00	0.00	0.20	0.49	0.69	0.00	0.69	Mar. 31	2.38
Apr. 5–Apr. 25	0.54	0.31	0.02	0.03	0.90	0.00	0.90	May 4	4.01
Apr. 26–May 30	0.25	0.23	0.10	0.19	0.77	2.28	3.05	June 8	0.96
May 31–July 4	0.19	0.14	0.25	0.15	0.73	5.70	6.43	July 6	2.70
July 5–Aug. 1	0.05	0.05	0.08	0.37	0.50	4.56	5.06	Aug. 3	3.85
Aug. 2–Sept. 5	0.04	0.04	0.00	0.05	0.13	5.70	5.83	Sept. 7	2.30
Sept. 6–Oct. 3	0.00	0.53	0.05	0.01	0.59	4.56	5.15	Oct. 12	4.89
Oct. 4–Oct. 31	0.06	0.25	0.05	0.05	0.41	1.14	1.55	Nov. 9	2.11
Nov. 1–Nov. 28	0.12	0.12	0.03	0.16	0.43	0.00	0.43	Dec. 16	2.61
Nov. 29–Dec. 31	0.00	0.00	0.05	0.12	0.17	0.00	0.17	Dec. 31	1.90[b]
Annual total	1.20	1.67	0.83	1.62	5.32	23.94	29.26		
% of Total input	4.1	5.7	2.8	5.5	18.2	81.8	100.0		

[a]May 11 value.
[b]Interpolated between Dec. 16 and Jan. 18.

or phosphorus, lake 227, 1970 and 1971. All data are in kilograms unless otherwise stated.

olumn (M)		Outflow (E)			Change in water column (△M)			Sedimentation (S=I-E-△M)	
TDP	Total	susp P	TDP	Total	susp P	TDP	Total	kg	% mass of susp P/day
0.89	3.79	0.29	0.09	0.38	−0.13	−0.35	+0.22	+0.09	+0.03
0.76	4.01	1.45	0.08	1.53	+0.17	−0.06	+0.11	−0.89	−0.57
0.93	4.13	0.36	0.05	0.41	−0.08	+2.62	+2.54	+3.15	+2.00
0.85	6.67	0.02	0.02	0.04	+2.25	+2.53	+4.78	+1.17	+0.59
3.10	11.44	0.00	0.00	0.00	−0.67	−2.70	−3.37	+9.16	+3.64
2.43	8.06	0.56	0.31	0.87	+0.84	−0.37	+0.47	+7.48	+3.92
3.27	8.54	0.82	0.41	1.23	−0.15	−1.66	−1.81	+1.21	+0.78
3.12	6.73	0.12	0.04	0.16	−0.53	−0.32	−0.85	−0.94	−1.23
2.59	5.88	0.03	0.01	0.04	+0.22	+0.06	+0.28	−0.28	−0.38
		3.65	1.01	4.66	+1.92	+0.45	+2.37	20.15	$\bar{x}$ = +1.20
		12.5	3.4	16.0			8.1	69.2	
2.81	6.16	0.00	0.00	0.00	−1.05	−0.97	−2.02	+2.71	+1.01
1.76	4.14	0.73	0.54	1.27	+1.78	+1.63	+3.41	−3.78	−5.63
3.54[a]	7.55	0.24	0.52	0.76	−2.58	+2.35	−0.23	+2.72	+3.13
6.36	7.33	0.44	0.33	0.77	+1.74	+1.46	+3.20	+2.46	+3.84
7.82	10.52	0.32	0.16	0.48	+1.15	+0.41	+1.56	+3.02	+3.29
8.23	11.08	0.01	0.01	0.02	−1.55	+0.72	−0.83	+6.64	+6.17
8.95	11.25	0.42	0.14	0.56	+0.55	−4.06	−3.51	+8.10	+8.05
2.85	7.74	0.31	0.28	0.59	−1.29	−2.78	−4.07	+5.03	+5.13
1.56	3.67	0.02	0.01	0.03	−0.38	+0.50	+0.12	+0.29	+0.42
1.18	3.79	0.00	0.00	0.00	−0.06	−0.71	+0.77	−0.63	−0.87
1.12[b]	3.02								
		2.49	1.99	4.47	−1.69	−1.45	−3.14	26.56	$\bar{x}$ = +2.58
		8.5	6.8	15.3				90.8	

TABLE 3. A summary of input, output, sedimentation, and changes in the water colum

Period 1970	Input (I)							Mass in wate	
	Inflows		Precipitation and dustfall		Total natural	Fertilizer	Total	Date	susp N
	susp N	TDN	susp N	TDN					
								Jan. 1	18.8
Jan. 1–Apr. 8	0.1	0.1	0.8	5.7	6.8	0.0	6.8	Apr. 9	22.8
Apr. 9–May 22	5.5	11.0	0.7	6.4	23.6	0.0	23.6	May 28	44.8
May 28–July 1	1.3	2.0	0.9	4.5	6.7	74.8	81.5	June 30	–
July 2–July 21	0.6	0.8	0.8	2.1	4.3	74.8	79.1	July 28	78.9
July 30–Sept. 2	0.0	0.2	0.4	2.4	3.0	74.8	77.8	Sept. 1	107.1
Sept. 3–Oct. 7	1.1	4.6	0.8	4.1	10.6	89.8	100.4	Oct. 6	36.4
Oct. 8–Nov. 11	2.8	5.8	0.5	3.3	12.4	0.0	12.4	Nov. 10	33.9
Nov. 12–Dec. 9	0.3	0.8	0.1	11.1	12.3	0.0	12.3	Dec. 9	25.9
Dec. 10–Dec. 31	0.1	0.1	0.1	1.0	1.3	0.0	1.3		
Annual total	11.8	25.4	5.1	40.6	81.0	314.2	395.2		
% of total input	3.0	6.4	1.3	10.3	20.5	79.5	100.0		
1971									
								Jan. 1	24.0
Jan. 1–Apr. 4	0.1	0.1	0.8	5.7	6.8	0.0	6.8	Mar. 31	18.2
Apr. 5–Apr. 25	2.0	7.3	0.1	0.5	9.9	0.0	9.9	May 4	–
Apr. 26–May 30	1.0	5.5	0.7	1.8	9.1	30.0	39.1	June 1	–
May 31–July 4	1.7	8.3	1.2	11.0	22.3	74.9	97.2	July 6	–
July 5–Aug. 1	0.3	2.6	0.4	1.6	4.9	59.9	64.8	Aug. 3	73.4
Aug. 2–Sept. 5	0.3	1.2	0.6	1.0	3.1	74.9	78.0	Sept. 7	88.0
Sept. 6–Oct. 3	0.5	3.7	0.5	1.3	6.0	59.9	65.9	Oct. 5	36.4
Oct. 4–Oct. 31	0.5	5.3	0.5	1.7	7.9	15.0	22.9	Nov. 9	19.3
Nov. 1–Nov. 28	0.6	0.6	0.3	2.0	3.6	0.0	3.6	Dec. 16	14.4
Nov. 29–Dec. 31	0.0	0.0	0.2	1.2	1.4	0.0	1.4	Dec. 31	14.1[c]
Annual total	7.0	34.6	5.3	27.8	75.0	314.6	389.6		
% of Total input	1.8	8.9	1.4	7.1	19.3	80.7	100.0		

[a]Apr. 9–July 21.
[b]Apr. 5–Aug. 1.
[c]Interpolated between Dec. 1 and Jan. 18.

for nitrogen, lake 227, 1970 and 1971. All data are in kilograms unless otherwise stated.

column (M)		Outflow (E)			Change in water column (ΔM)			Sedimentation (S=I-E-ΔM)	
TDN	Total	susp N	TDN	Total	susp N	TDN	Total	kg	% mass of susp N/day
87.4	106.2	1.6	12.5	14.1	+4.0	+37.6	+41.6	−48.9	−2.40
125.0	147.8	6.1	69.1	75.2		insufficient data		–	–
–	–	3.4	7.0	10.4		insufficient data		–	–
–	–	0.3	0.5	0.8	+56.1[a]	−6.3[a]	+49.3[a]	+48.5[a]	+0.89
118.7	197.6	0.0	0.0	0.0	+28.2	+10.2	+38.4	+39.4	+1.18
128.9	236.0	4.9	9.3	14.2	−70.7	+38.5	−32.2	+118.4	+4.67
167.4	203.8	5.4	42.0	47.4	−2.5	+40.6	+38.1	−73.1	−5.94
208.0	241.9	0.7	5.2	5.9	−8.0	+65.0	+57.0	−103.7	−12.39
273.0	298.9	0.2	1.3	1.5	−1.9	−20.8	−22.7	+22.5	+4.10
		22.6	146.9	169.5	+5.2	+164.8	+170.0	+3.1	+0.02
		5.7	37.2	42.9				0.8	
252.2	276.2	0.0	0.0	0.0	−5.8	−143.7	−149.5	+156.3	+7.88
108.5	126.7	5.9	29.1	35.0		insufficient data		–	–
–	–	4.3	33.3	37.6		insufficient data		–	–
116.4	–	4.8	6.7	11.5		insufficient data		–	–
136.2	–	4.7	3.8	8.5	+55.2[b]	+38.6[b]	+93.8	+24.9	+0.46
147.1	220.5	0.1	0.2	0.3	+44.6	−10.8	+3.8	+73.9	+2.62
136.3	224.3	3.0	1.8	4.8	−51.6	+31.3	−20.3	+81.4	+4.67
167.6	204.0	4.0	9.9	13.9	−17.1	−10.6	−27.7	+36.7	+4.71
157.0	176.3	0.1	0.6	0.7	−4.9	+12.0	+7.1	−4.2	−0.86
169.0	183.4	0.0	0.0	0.0	−0.3	−11.8	−12.1	+13.5	+2.96
157.2[c]	171.3								
		26.9	85.4	112.3	−9.9	−95.0	−104.9	+382.5	−2.91
		6.9	21.9	28.8				98.2	

TABLE 4. A summary of input, output, sedimentation, and changes in the water column

Period 1970	Input (I)					Mass in water column (M)			
	Inflows		Precipitation and dustfall						
	susp C	DIC	susp C	DIC	Total[a]	Date	susp C	DIC	Total[a]
Jan. 1–Apr. 8	0	0	17	15	32	Jan. 22	177	325	502
Apr. 9–May 27	28	1	17	9	55	Apr. 9	172	1440	1612
May 28–July 1	10	0	8	0	18	May 28	232	690	922
July 2–July 29	1	0	4	0	5	July 7	695	474	1169
July 30–Sept. 2	0	0	3	0	3	July 28	642	479	1119
Sept. 3–Oct. 7	7	0	8	0	15	Sept. 8	740	643	1383
Oct. 8–Nov. 11	14	1	7	0	22	Oct. 13	283	1184	1467
Nov. 12–Dec. 9	1	0	4	3	8	Nov. 10	253	813	1066
Dec. 10–Dec. 31	0	0	2	5	7	Dec. 9	209	969	1178
Annual total	61	2	70	32	165				
% of total input	1.8	0.0	2.1	1.0	5.0				
1971									
Jan. 1–Apr. 4	0	0	28	7	35	Jan. 1	191	1095	1286
Apr. 5–Apr. 25	20	70	2	0	92	Mar. 31	146	1341	1487
Apr. 26–May 30	62	46	11	1	120	May 4	272	884	1156
May 31–July 4	11	36	15	0	62	June 1	248	646	894
July 5–Aug. 1	4	18	6	0	28	July 6	695	1311	2006
Aug. 2–Sept. 5	5	17	3	0	25	Aug. 3	427	1283	1710
Sept. 6–Oct. 3	6	46	1	0	53	Sept. 7	735	1067	1802
Oct. 4–Oct. 31	10	64	5	0	79	Oct. 5	317	1306	1623
Nov. 1–Nov. 28	7	33	14	4	58	Nov. 9	241	963	1204
Nov. 29–Dec. 31	0	0	9	2	11	Dec. 16	281	898	1179
						Dec. 31	205[b]	993[b]	1198
Annual total	125	330	94	14	563				
% of Total Input	7.0	18.4	5.2	0.7	31.4				

[a]Susp C + DIC.
[b]Interpolated between Dec. 16 and Jan. 18.
[c]R = Ratio of mass of susp C to susp P in lake; Se = Sedimentation rate of phosphorus, kg.

for carbon, lake 227, 1970 and 1971. All data are in kilograms unless otherwise stated.

Outflow (E)			Change in water column (△M)			Sedimentation ($S_c=S_p(R^e)$)		
susp C	DIC	Total[a]	susp C	DIC	Total[a]	kg	% of mass of susp C/day	Invasion (A=E+△M+S-I)
94	13	107	−5	+1115	+1110	+10	+0.06	+1195
184	53	237	+60	−750	−690	−107	−1.10	−615
7	19	26	+463	−216	+247	+234	+1.44	+489
0	2	2	−53	+3	−50	+120	+0.64	+67
0	0	0	+98	+166	+264	+997	+4.01	+1424
34	51	85	−457	+541	+84	+512	+2.86	+666
42	45	87	−30	−371	−401	+90	+0.96	−246
24	6	30	−44	+156	+112	−63	−0.97	+71
6	1	7	−18	+126	+108	−16	−0.36	+92
461	190	581	+14	+770	+784	+1777	+1.29	3143
13.9	5.7	17.5				53.7		95.1
								Total input[a] 3308
0	0	0	−45	+246	+201	+192	+1.21	+358
102	42	144	+126	−457	−331	−246	−5.60	−341
91	14	105	−24	−238	−262	+147	+1.62	−30
100	41	141	+447	+665	+1112	+167	+1.01	+1358
28	37	65	−268	−28	−296	+222	+1.41	−37
2	1	3	+308	−216	+92	+518	+2.55	+588
18	11	29	−418	+239	−179	+524	+3.56	+321
63	50	113	−76	−343	−419	+386	+4.94	+1
2	1	3	+40	−65	−25	−271	−3.58	−351
0	0	0	−76	+95	+19	−94	−1.21	−437
406	197	603	+14	−102	−88	+1545	+1.38	1230
22.6	11.0	33.6				86.2		68.6
								Total input[a] 1793

even though waters are supersaturated with gaseous CO_2 with respect to the atmosphere.

Once ice leaves the lake in April or May, much of the winter accumulation is lost to the atmosphere. This decrease in DIC continues until algal demand for CO_2 is sufficiently great to lower P_{CO_2} of the epilimnion to less than atmospheric values. This usually occurs in late May or early June, after fertilization with P and N has been in progress for a few weeks. For the next 2–4 months, depending on weather conditions, the lake accumulates CO_2 invading from the atmosphere, as well as from decomposition. Once cool weather begins to erode the thermocline at fall overturn, sweeping hypolimnetic water with high DIC concentrations back into contact with the atmosphere, the lake again loses DIC. Some loss also was evident during cool weather in July of 1971 and August of 1972, when algal blooms were decreasing rapidly.

Altogether, the accumulation of DIC indicates an excess of incoming CO_2 during summer over that lost in spring and fall. The lake therefore appears to be correcting its own carbon deficit, in response to the increased input of phosphorus and nitrogen.

General Discussion

Much concern has been shown in North America for the role that carbon plays in eutrophication. In the USA, this concern has delayed critically needed phosphorus control, in spite of the fact that such control is favored by scientific opinion.

Virtually all of the "evidence" for carbon limitation is irrelevant to the problem of controlling algal blooms. Laboratory experiments with algal cultures or unfiltered lake water experiments have been previously criticized by D. W. Schindler (1971). Field experiments and observational evidence are discussed below, since comparison with similar experiments and observations in lake 227 clearly show their shortcomings.

Kuentzel (1969) calculated the amount of carbon required for producing the algal bloom observed by Mackenthun et al. (1968) in Lake Sebasticook, compared it to concentrations of DIC, and concluded that the nececessary carbon would have to be supplied by DOC. It is clear from our results that such calculations, without regard for inputs of DIC from atmosphere and other sources, can be totally misleading. In lake 227, DIC concentrations in the epilimnion in midsummer were not sufficient to support even one day's algal production (Schindler and Fee 1973). DOC concentrations did not change, and the supply of DOC to the lake were negligible, therefore it is unimportant as a source of DIC. In larger, more turbulent lakes, carbon could be supplied by the atmosphere at even higher rates, even if P_{CO_2}'s are lower. A dynamic process, algal growth, is compared by Kuentzel to a static measurement, carbon concentration, i.e. the units being compared are mg/liter per unit time on one hand and mg/liter on the other. The comparison is therefore unsound in theory as well as misleading.

Kerr et al. (1970) and King (1970) have described water bodies where the supply of carbon was insufficient to allow phytoplankton standing crops to exercise their full potential production. In such situations, addition of carbon is likely to stimulate primary production and algal growth greatly (see discussion by Goldman et al. 1971, p. 32–33).

Our bottle bioassay studies in lake 227 have given similar results (D. W. Schindler 1971). Further investigation has shown that photosynthesis was extremely high in the lake at dawn, when DIC was adequate due to overnight increases of CO_2 by respiring plankton and atmospheric invasion. By midmorning, however, production was severely curtailed, due to an extreme shortage of DIC (Schindler and Fee 1973). The reader should be reminded that this situation in lake 227 exists where evidence that phosphorus and nitrogen have caused the increase in standing crop is irrefutable. The standing crop of phytoplankton in midsummer is near the maximum theoretically possible per unit area of epilimnion (200–300 mg/m^2 of chlorophyll-*a*; Talling et al. 1973).

The answer to this apparent paradox has been simply summarized by O'Brien (1972). He pointed out that responses of phytoplankton to limiting factors may be of two types, change in standing crop[2], as in the case of lake 227, or change in growth or production rate, as observed in bottle-bioassay experiments. Both responses may occur simultaneously. Thus, in a system like lake 227, addition of carbon might greatly stimulate rates of phytoplankton reproduction, while the ultimate "carrying capacity" of the system could not exceed the limits set by available phosphorus. This interpretation is supported by other experiments conducted in lake 227 and in lake 304, where addition of dissolved inorganic and organic carbon to large enclosures in the lake did not increase the standing crop of algae over that due to addition of phosphorus and nitrogen alone (Schindler 1971; Schindler et al. 1971, and unpublished data). While both phosphorus and nitrogen were required to produce large standing crops of phytoplankton, additions of the

[2]Yield in O'Brien's paper refers to equilibrium standing crop, which is obvious from his figure 1. We also suggest that supply rather than concentration of nutrient would be more appropriate.

elements singly revealed that phosphorus always caused some increase in standing crop, while addition of nitrogen alone never elicited a response. The key to controlling algal standing crops in lake 227 and similar waters lies in controlling supplies of the element phosphorus.

It is clear from our results that ^{14}C bioassays (Goldman 1972) have rather limited use as indicators of nutrients responsible for eutrophication, if the ecosystem under surveillance has already been modified to some undetermined extent. As our results show, the nutrients found to be limiting in such circumstances may not be the same ones which caused alteration of the system in the first place. Thus, in bottle bioassays conducted after several weeks of fertilization with phosphorus and nitrogen, DIC was found to be the most stimulatory element. Yet it is clear that the key to controlling phytoplankton in the lake is not DIC. If lake 227 were a lake with an unknown history of fertilization, the wrong conclusions might be drawn from such bioassays regarding nutrient control. It is possible that such errors in interpretation may already be affecting one environment of major concern, the eastern U.S. coast. In a review of recent work, Ryther and Dunstan (1971) have logically concluded that nitrogen is usually the limiting factor in such waters, and that increasing supplies of nitrogen would cause further deterioration of water quality. The same logic cannot, however, be extrapolated backward in time, or applied when nutrient control to reduce rather than check eutrophication problems is desired. The rivers and estuaries discussed by Ryther and Dunstan have received inputs of nutrients for many decades in which the ratio of phosphorus to nitrogen is far higher than in natural inputs.[3] It is therefore possible that phosphorus was limiting to the aquatic flora of these streams in the past, only to be supplanted by nitrogen when cultural sources exceeded demands for the former element.

Accumulation of undesired quantities of algae, which is the common man's measure of eutrophication, is the result of a complex of causal factors. Standing crop of algae can be made to accumulate by reducing sedimentation and grazing by zooplankton just as surely as by an increase in production. For example, Haney (1970) has shown that zooplankton may "turn over" the entire standing crop of phytoplankton in less than a day under some conditions. Yet D. W. Schindler (1968) and J. E. Schindler (1971) have found that water-bloom forms of blue-green algae are less ingestible and digestible by zooplankton than other algal groups. This could lead to accumulation of unwanted algal species without any alteration of production rates. The unconsidered reapplication of techniques designed primarily for information gathering to problems where cause and effect relations are to be tested as rigorous hypotheses must be avoided at all costs. The resulting confusion and misinformation can lead only to a decline in credibility of aquatic ecology as an effective force in solving environmental problems.

[3]North American sewage effluents usually have P:N in a ratio of about 1:4 to 1:5 by weight, while most natural inputs would range between 1:15 and 1:30.

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Drs E. J. Fee, A. L. Hamilton, D. R. S. Lean, K. Patalas, and J. R. Vallentyne provided useful criticism of the manuscript. Miss C. Boyce drew the figures. M. P. Stainton and co-workers provided analyses of suspended C, N, P, and major cations.

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A Syringe Gas-Stripping Procedure for Gas-Chromatographic Determination of Dissolved Inorganic and Organic Carbon in Fresh Water and Carbonates in Sediments

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STAINTON, M. P. 1973. A syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments. J. Fish. Res. Board Can. 30: 1441–1445.

A simple, rapid method for determining dissolved inorganic carbon in water is described. A 20-cm³ sample of water is drawn into a 50-cm³ polypropylene syringe and acidified by injection of 1 cm³ of dilute sulphuric acid. Twenty-nine cubic centimeters of helium at atmospheric pressure is injected into the syringe followed by 10 sec of manual agitation to partition CO_2 between gas and liquid phase. The gas phase containing 60% of CO_2 from the sample is then analyzed by gas chromatography. This method has been used to determine dissolved inorganic and organic carbon in Canadian Shield waters and to determine total carbonates in sediments.

STAINTON, M. P. 1973. A syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments. J. Fish. Res. Board Can. 30: 1441–1445.

L'auteur décrit une méthode simple et rapide de dosage du carbone inorganique dissous dans l'eau. Un échantillon de 20 cc d'eau est prélevé dans une seringue de polypropylène de 50 cc et acidifié par injection de 1 cc d'acide sulfurique dilué. On injecte ensuite dans la seringue 29 cc d'hélium à la pression atmosphérique et on agite manuellement pendant 10 sec, jusqu'au partage du CO_2 entre la phase gazeuse et la phase liquide. La phase gazeuse, contenant 60% du CO_2 de l'échantillon, est ensuite analysée par chromatographie en phase gazeuse. Cette méthode a été utilisée pour le dosage du carbone inorganique et organique des eaux du Bouclier canadien et des carbonates totaux des sédiments.

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SEVERAL techniques exist for the determination of inorganic carbon in fresh water. Few of these, however, are applicable to the soft waters found in lakes of the Precambrian Shield where concentration of dissolved inorganic carbon (DIC) are often under 100 μmoles per liter. Problems with the widely used acid titration were discussed by Schindler and Holmgren (1971). The gravimetric technique of Goldman (1963) and the dialysis concentration technique of Johnson et al. (1970), while effective, require prohibitively large quantities of sample and are quite time consuming. This work describes a modified gas-chromatographic technique for analysis of DIC.

Previously reported gas-chromatographic techniques suffered from problems arising from the dynamic nature of the sparging systems employed. Swinnerton et al. (1962) reported a technique involving injection of a small volume of acidified sample into a flowing gas stream. CO_2 was stripped from the sample by the gas stream and swept directly onto the chromatographic column. This was a nonequilibrium, dynamic system since CO_2 stripping was not instantaneous. The resulting peaks were broad and asymmetric, requiring area integration for quantitative work. The limit of detection for CO_2 was about 25 μM.

An improvement to the above approach was effected by Williams and Miller (1962), who employed a system that achieved dynamic equilibrium between acidified water and helium run countercurrently through a series of rotating Mylar discs. Samples of the equilibrated gas phase were injected onto the chromatographic column via a sample loop valve. While this method has been found to give us excellent quantitative data, the need to establish a dynamic equilibrium for each sample analyzed is consumptive of time (10 min per sample) and sample (500 cm³).

The use of a syringe as a reaction chamber to establish a static equilibrium of dissolved gas between liquid and helium has been reported by

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McAuliffe (1971) for determining hydrocarbons in water and Stainton (1971) for determining mercury in tissue. The method described below uses a 50-cm^3 polypropylene syringe as a reaction chamber to establish static equilibrium between 20 cm^3 of sample, 1 cm^3 of dilute acid, and 29 cm^3 of helium. The analytical system is simple, inexpensive, and provides sensitivity (detection limit 1 μM CO_2) sufficient for most studies of CO_2 in water. Precision is excellent with a coefficient of variation of 0.73% at the 100 μM level. The method has been successfully employed to measure DIC in fresh water, dissolved organic carbon in fresh water (following a wet oxidation by the method of Menzel and Vaccaro (1964)) and total carbonates in sediments (following acid digestion at elevated temperatures).

Materials and Methods

Dissolved Inorganic Carbon

Water samples were collected in 50-cm^3 polypropylene syringes, directly from a 3-liter van Dorn sampler. Syringes were capped immediately upon filling and transported to the laboratory. All but 20 cm^3 of sample was expelled from the syringe for conductance, temperature, and *p*H measurements. The remaining 20-cm^3 sample was acidified with 1 cm^3 of 2N sulphuric acid injected into the sample syringe from a Cornwall repeating syringe via a double female luer coupling. The syringe containing the acidified sample was then attached to the three-way valve shown in Fig. 1 and helium injected at low pressure until the plunger reached the 50-cm^3 graduation. The syringe was quickly capped and agitated vigorously by hand for 10 sec. If necessary, groups of samples processed to this stage could be left standing for several hours while the gas chromatograph (Fisher Hamilton Model 29) was calibrated.

All water used in this study was from a Millipore "Super Q" system. The effluent from this system was typically less than 5 μM in CO_2 and less than 50 μM in dissolved organic carbon.

Using the stock bicarbonate standard (0.8401 g dry $NaHCO_3$/liter of "Super Q" water), 100 cm^3 quantities of standards were prepared fresh daily to cover the range from 100 to 2000 μmoles CO_2/liter. Blanks and standards were stored in stoppered 100-cm^3 volumetric flasks until used. Using a 50-cm^3 syringe with the plunger fully depressed, 20 cm^3 of blank or standard solution were drawn up taking care not to include bubbles of air

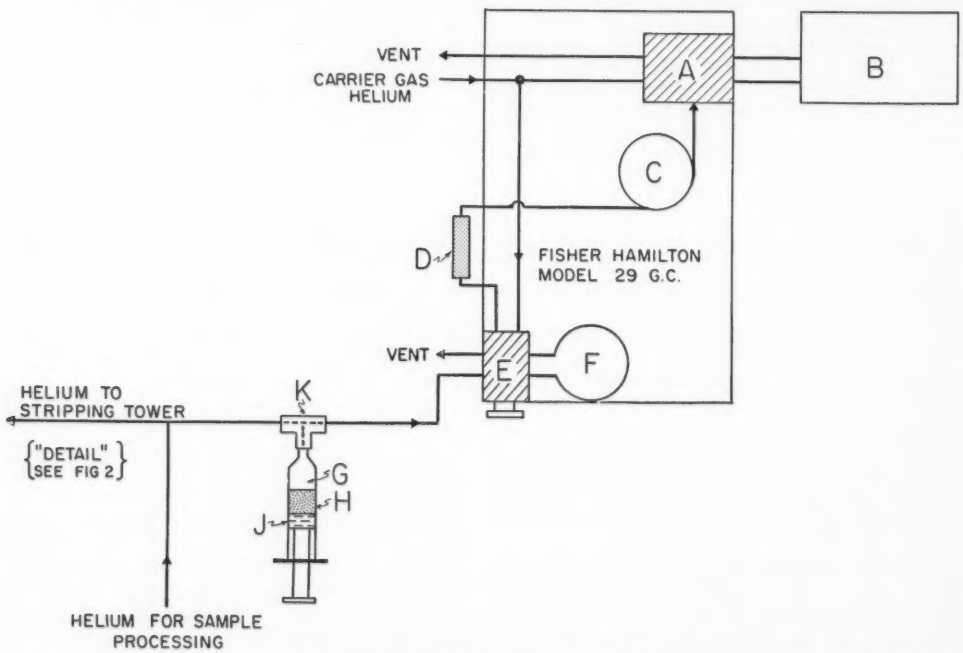

FIG. 1. Analytical system for CO_2 determination. A, Detector — thermal conductance type; B, Recorder — 1 mv full scale — 0.2 inches per min; C, Chromatographic column — 4 ft × ⅛ inch ID — Poropak Q packing; D, "Drierite" drying column; E, Sample loop valve; F, Sample loop — 1–5 cm^3 depending on sensitivity needed; G, Helium CO_2 mixture; H, Acidified sample; J, Syringe plunger; K, Three-way valve.

with the liquid. Blanks and standards were acidified and partitioned with helium as outlined above for samples.

A syringe containing the acidified sam le partitioned with helium was attached to the three-way valve. With the three-way valve in position for injection through the sample loop valve, 15 cm^3 of the gas phase in the syringe was expelled and the sample loop valve quickly switched to inject the helium–CO_2 mixture onto the chromatographic columns. This procedure was repeated for blanks, standards, and samples.

Peak height and attenuation required for blanks, standards, and samples were recorded. A scale factor (μmoles per liter per chart unit) was calculated from standard peak heights corrected for blank. Sample peak heights were simply multiplied by this scale factor to give μmoles CO_2 per liter, there being no sample blank.

Dissolved Organic Carbon

The procedure outlined is essentially that of Menzel and Vaccaro (1964). Modifications have been made to utilize the economy, sensitivity, and linearity of a gas-chromatographic analysis for CO_2.

A 50-cm^3 sample of water previously passed through a Whatman GF/C filter was added to the stripping tower diagramed in Fig. 2. Helium flow was adjusted to produce vigorous bubbling through the filter frit and 0.1 cm^3 of analytical reagent grade perchloric acid (60% w/w; S.G. 1.54) was added. The acidified sample was bubbled for 10 min to remove inorganic carbon. The drain tube was then flushed with 5 cm^3 of sample and 25 cm^3 of sample run off into a preignited (550 C for 8 hr) glass ampoule containing 0.1 g of analytical reagent grade potassium persulphate. Care was taken to avoid percolating the sample through air, the drain tube being held beneath the liquid surface in the ampoule during filling. The neck of the ampoule was then sealed with a propane oxygen flame taking care not to entrap flame combustion gases in the ampoule. Sealed ampoules were then autoclaved at 121 C for 1 hr to combust organic matter to CO_2. Samples could be stored indefinitely at this stage.

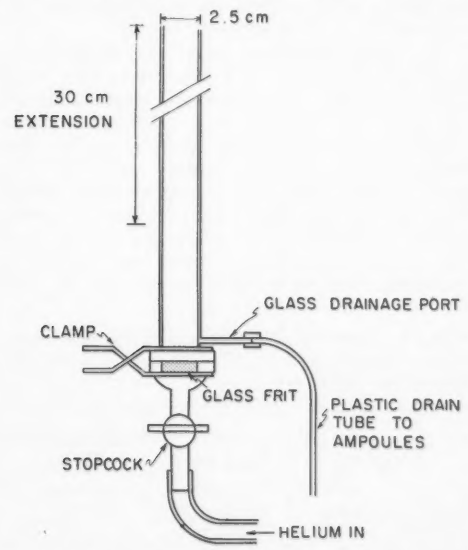

Fig. 2. CO_2 stripping tower from modified 25-mm Millipore filtration manifold.

Samples wet oxidized as above and cooled to room temperature were quickly transferred from opened ampoules to 50-cm^3 polypropylene syringes. A wide bore cannula was used to permit rapid transfer of liquid with minimum degassing. Samples were then analyzed for CO_2 content as outlined previously for inorganic carbon.

The method of sample transfer from ampoule to syringe permits loss of any CO_2 in the gas space in the ampoule neck. This appears to be a fairly constant 4% of the CO_2 level of the sample. For this reason and as a check on the effectiveness of oxidation it was found necessary to calibrate the analyses by treating glucose standards and low carbon water by the same procedure as samples. A scale factor was computed from the glucose standard peak height, corrected for low carbon water blank. No blank correction was necessary for samples.

Carbonate in Sediments

Appropriate quantities of dried samples of calcite (analytical reagent grade $CaCO_3$) and dolomite (99.9% $MgCO_3 \cdot CaCO_3$) standards were weighed to the nearest 10 μg and placed in preignited (550 C for 8 hr) glass ampoules. The quantity weighed provided between 0.5 and 50 μmoles of carbonate and bicarbonate carbon per ampoule. Twenty-five cubic centimeters of low carbon water were then added to the ampoules, followed by 50 μliters of concentrated sulphuric acid and the ampoules sealed immediately. Sealed ampoules were autoclaved for 1 hr at 121 C and liberated CO_2 determined as for dissolved inorganic carbon. Calibration was via calcite and dolomite standards analyzed by the same procedure as samples.

Results and Discussion

Dissolved Inorganic Carbon

A series of six replicates of DIC at the 100-μM level was found to have a coefficient of variance of 0.76%. Sensitivity was found to be about 0.3 μmoles CO_2 per liter per μv with response being linear up to 10,000 μM. The partitioning coefficient of CO_2 between sample and helium was measured to be 1.04. With the ratio of gas to liquid used (29:21), 60% of available CO_2 was partitioned from liquid to gas phase.

Samples held for 4 hr after the 10-sec agitation showed no detectable loss or gain of DIC from

capped syringes. It was therefore possible to batch process samples, usually at the rate of one analysis every 2 min.

Although sensitivity of the method can be enhanced by increasing the sample loop volume of the gas chromatograph and increasing the ratio of sample liquid to helium, the conditions outlined above were quite adequate for analysis of Shield waters.

Dissolved Organic Carbon

The method as outlined was tested for precision using six replicates of a glucose solution 1000 μM in carbon. Sensitivity was of course the same as for inorganic carbon; however, precision deteriorated with a coefficient of variance of 2.3%. Blanks for the low carbon water used varied from 30 to 50 μmoles C/liter with only 1–2 μmoles of this being a reagent blank. This reagent blank was ignored in subsequent calculations of dissolved organic carbon in samples.

Recovery was tested in two ways, first with aqueous solutions of known organic compounds and later with ^{14}C-labelled algal metabolites and excretory products.

Results of recovery tests using known compounds appear in Table 1 with recoveries expressed relative to both bicarbonate and glucose standards carried through the same wet oxidation. For the rather limited range of compounds tested, recoveries appear to be acceptable.

To obtain an indication of how the method would cope with naturally occurring freshwater organics, a solution of ^{14}C labelled organics was prepared by growing a culture of *Anabaena flos-aqua* in a closed system for 15 days with ^{14}C bicarbonate as the only carbon source. The filtrate through a 0.22-μ membrane filter was acidified and activity measured by liquid scintillation counting. The acidified filtrate was then bubbled with helium for 30 min to remove $^{14}CO_2$, and the activity of the stripped filtrate measured again, with 64% of solution's original activity remaining, presumably in dissolved organic compounds. The filtrate was then wet oxidized at various dilutions as described above, bubbled with helium for 30 min and remaining activity measured. The amount of activity remaining after this treatment was an indication of the amount of labelled organic matter not oxidized to CO_2. At the 7500 μM carbon level 1% of ^{14}C activity remained, while at the 1500 μM level 0.1% remained.

These results are not a conclusive demonstration of oxidation efficiency as the 2-week labelling period may not have been sufficient to label the more refractory compounds present in natural waters.

Table 1. Recovery of carbon from some pure organic compounds by gas chromatography following wet oxidation. All tests run at the 1000 μM level.

Compound	% recovery relative to bicarbonate standard	% recovery relative to glucose standard
Nitrilotriacetic acid (as tri sodium salt)	98.4	99.0
Oxalic acid	95.4	96.0
Tartaric acid	98.9	99.5
Dextran	99.6	100.3
Urea	94.4	95.0
Sucrose	98.3	98.9
Glucose	99.4	100.0

Carbonate in Sediments

Six replicates of calcium carbonates, dolomite, and dried sediment from Lake Winnipeg treated as outlined in the method, gave the recoveries and precisions listed in Table 2. Method specificity was tested by carrying samples of dried humic extract and acid leached sediment through the analysis. No detectable breakdown of organic carbon to CO_2 was observed. Apparently the acid conditions and temperature employed are not sufficient to oxidize sediment organics.

The above techniques have been found to be applicable in many field situations. The small sample size, low contamination, speed, and sensitivity of the analysis have permitted accurate analyses where it was not possible before. The methods for dissolved organic and inorganic carbon allow samples to be taken with a syringe from sharply stratified lakes or tanks at levels defined to within a few centimeters. The method for inorganic carbon has been found useful in estimating benthic primary production by DIC

Table 2. Recovery efficiency and precision for gas-chromatographic analysis of sedimentary calcium carbonate and dolomite.

Compound	% recovery	Precision (rel. SD) (n=6)
Calcium carbonate	99.5	2.8%
Dolomite	101.2	1.2%
Sediment — Lake Winnipeg	–	0.4%

uptake (Schindler et al. 1973) and for measuring diurnal CO_2 fluctuations.

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I thank D. W. Schindler and F. A. J. Armstrong for critical comments, and R. V. Schmidt for field testing the methods.

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A Numerical Model for Determining Integral Primary Production and its Application to Lake Michigan[1,2]

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FEE, E. J. 1973. A numerical model for determining integral primary production and its application to Lake Michigan. J. Fish. Res. Board Can. 30: 1447–1468.

A computer-based model for determining production by phytoplankton, integrated over depth and over an arbitrary time interval, is described. The solution incorporates light inhibition and uses the actual distribution of surface irradiance for the time interval of interest, since it is not possible to predict the detailed nature of cloudiness. Statistical procedures for estimating the model parameters from experimental data relating the rate of carbon uptake to irradiance are described. The model is applied to data collected from May 27, 1970 through February 3, 1971 from Lake Michigan.

Integral primary production was bimodal at inshore and offshore stations with minimum production in midsummer and winter. There was great daily variability of integral production, due solely to variation of light. From this it is inferred that occasional in situ measurements would give a very poor knowledge of true seasonal trends.

The model output was verified by performing two in situ experiments. The agreement was better than 95% on both dates. The model makes it possible to estimate integral primary production on a routine basis in large water bodies with well-mixed photic zones.

FEE, E. J. 1973. A numerical model for determining integral primary production and its application to Lake Michigan. J. Fish. Res. Board Can. 30: 1447–1468.

L'auteur décrit un modèle pour ordinateur capable de mesurer la production primaire du phytoplancton intégrée sur la profondeur et sur un intervalle de temps arbitraire. Etant donné qu'il est impossible de prédire le degré exact de nébulosité, l'équation tient compte de la suppression de la lumière et utilise la distribution réelle du rayonnement en surface pour l'intervalle de temps qui nous intéresse. L'auteur décrit la méthode statistique utilisée pour estimer les paramètres du modèle à partir de données expérimentales reliant le taux de captation du carbone au rayonnement. Il applique le modèle à des données recueillies dans le lac Michigan du 27 mai 1970 au 3 février 1971.

La production primaire intégrale est bimodale aux stations côtières et hauturières, le minimum de production se trouvant à la mi-été et en hiver. Le minimum d'été résulte de l'épuisement des substances nutritives dans l'épilimnion; celui d'hiver est causé par l'insuffisance de lumière. La production intégrale subit de fortes variations journalières causées exclusivement par des fluctuations de la lumière. On en déduit que des mesures occasionnelles in situ donneraient une pauvre idée des tendances saisonnières réelles.

L'auteur a vérifié le rendement du modèle lors de deux essais in situ. L'accord a été de plus de 95% aux deux dates. Le modèle rend possible l'estimation, de façon routinière, de la production primaire intégrale dans les grandes nappes d'eau à zones photiques bien mélangées.

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THE popular in situ method for measuring phytoplankton production is inadequate for estimating the production of large water bodies. The number of stations that can be processed in a day is far too small to provide synoptic information. Futher, since natural light is used, experiments can be performed only during a brief part of the day — a wasteful consequence when ship time is costly or large areas are being studied.

Indeed, the in situ method is of limited usefulness even in the study of small lakes. Day-to-day variability of primary production is often so great

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(Rodhe et al. 1958) that daily measurements are needed to reliably estimate annual or seasonal production rates. This is clearly impractical with the in situ method. Futher, since the actual variations of surface irradiance are not analytically incorporated, the technique provides no rational approach for interpolating between observations separated in space or time. Also, technical complications associated with the use of ^{14}C as a tracer of carbon uptake, for example, extracellular excretion and respiration, confine incubation times to short intervals, usually 4–6 hr (Vollenweider et al. 1961). It is difficult to realistically extrapolate these short-term rates, which usually cover only a short part of the daylight period, to daily rates (Vollenweider 1970). Because natural light is used, it is not possible to assign an observed rate of photosynthesis to a specific irradiance. For example, the light at a given depth may be at inhibitory levels at the beginning of an experiment and at suboptimum levels at the end. It is impossible to define the physiological characteristics of the phytoplankton under such conditions because of the highly non-linear relation between photosynthesis and light. Finally, since the in situ method is entirely empirically based, it has little predictive power.

As a result of these problems, knowledge of the productivity of large water bodies has remained scanty. A major purpose of this paper, therefore, is the formulation of a methodology to cope with the difficulties of estimating phytoplankton production in large bodies of water such as the Laurentian Great Lakes and in the oceans. The new approach will also be useful for studying the detailed aspects of primary production in smaller lakes.

Model Development

In the model that is developed here, phytoplankton are treated as a single physiological entity. the response of a unit volume of sample — which is the quantity of practical importance — is modeled; and the variations in species composition are ignored.

Four assumptions are needed to derive an analytical solution of the integral photosynthesis equation suitable for computer analysis. In order to compute the depth integral, phytoplankton are assumed to be uniformly distributed in a vertically isothermal euphotic zone which is optically homogeneous. To extend the integration over time, it is further assumed that the physiological properties of the phytoplankton do not vary from sunrise to sunset. The logical consequences of these assumptions are the model developed below. Appendix 1 summarizes the symbols used in the treatment.

Integration of Photosynthesis over Depth and Time

Vollenweider (1965) proposed an equation,

$$P = P_{max} \frac{I/I_k}{\sqrt{1 + (I/I_k)^2}} \cdot \frac{1}{[1 + (aI/I_k)^2]^{n/2}}, \quad [1]$$

to relate production rate (P) to irradiance (I). This equation provides a good description of empirical results from cultures and field populations. An analytical result for computing daily integral primary production was derived from equation [1] by Fee (1969). This result is

$$\int_{-\lambda/2}^{\lambda/2} \int_{o}^{\infty} P dz dt = \sum\sum P = \frac{P_{opt}\,\lambda}{\epsilon} \left[\frac{\delta}{2} \int_{-1}^{1} \int_{0}^{I_o(x)/\delta I'_k} \times \frac{1}{[(1+y^2)(1+a^2y^2)^n]^{1/2}}\, dy\, dx \right]. \quad [2]$$

Depending upon the form of $I_o(x)$, the solution of the bracketed term may be tabulated or solved for an arbitrary empirical function. In the general case, i.e. when $I_o(x)$ is an empirical function, equation (2) is analytically intractible and the answers must be obtained numerically with a computer or by mechanical integration.

It is assumed that P_{opt}, ϵ, I'_k, λ, $I_o(x)$, a, and n will be measured or otherwise predicted. The techniques for estimating each of these parameters are explained below. The only remaining quantity that must be known to complete the solution is δ, which is uniquely determined by a and n. This may be found by differentiating equation [1] with respect to I and setting the resulting expression equal to zero. The solution, hereafter called I_{opt}, is the irradiance at which the optimum or peak on the photosynthetic rate vs. irradiance curve is attained. Substitution of I_{opt} into equation [1] in place of I gives P_{opt}, and δ follows from the relation $\delta = P_{max}/P_{opt}$. Complete details are presented by Fee (1971a); the result is

$$I_{opt} = \left[\frac{(1-n)a + \sqrt{a^2(n-1)^2 + 4n}}{2an} \right]^{1/2}$$

and thus

$$\delta = [(1 + I_{opt}^2)(1 + a^2 I_{opt}^2)^n]^{1/2}/I_{opt}.$$

A digital computer program was developed to solve equation [2] numerically. This program is

written in Fortran IV and is presented in detail. by Fee (1971b). The parameters P_{opt}, I'_k, λ, ϵ, a, and n are required as input. The function $I_o(x)$ must also be specified digitally. Both $I_o(x_o)$ and λ are best determined empirically with a solarimeter because of the impossibility of predicting variations in cloudiness. The parameters a and n may be estimated with a second computer program (Fee 1971b) or may be assigned values by comparison of experimental P vs. I curves with the family of curves presented by Fee (1969). I'_k is estimated either by the method of Vollenweider (1960) for in situ curves (Fig. 2) or read directly off a plot of P vs. I from incubator experiments (Fig. 1)

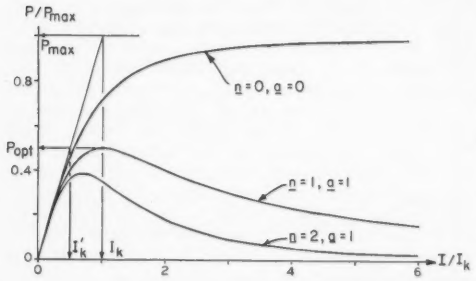

FIG. 1. Vollenweider's photosynthesis equation, showing the three cases whose photosynthesis vs. depth curves are analytically integrable. The model parameter I_k is the irradiance at which extrapolation of the nearly linear portion of the P/I curve would intersect the asymptote $P=P_{max}$, and I_k' the irradiance at which the extrapolated line would intersect $P=P_{opt}$.

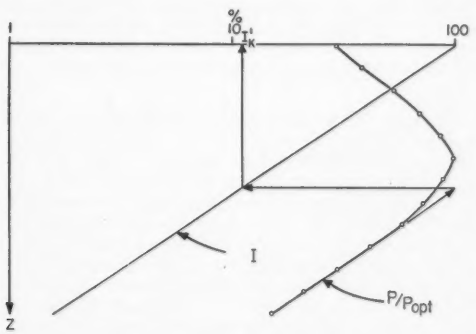

FIG. 2. Semilogarithmic plot of relative photosynthesis (P/P_{opt}) vs. depth in an ideal case. Also plotted is the extinction of light (I). Note the parallel and linear nature of these curves at depths below the optimum depth for photosynthesis.

Note (Fig. 2) that the linearity of P/P_{opt} vs. depth on a semilogarithmic plot is a criterion for testing the vertical homogeneity and the slope of this line is the extinction coefficient. The computer program used for estimating a and n also finds the least squares estimate of I'_k given the initial estimate.

The use of a single extinction coefficient, ε, to characterize light penetration appears to be an oversimplification, since the extinction varies (Hutchinson 1957) with wavelength. However, the extinction over depth of the integral of all visible light energy is usefully described by a single exponential coefficient for depths below 1 m (Vollenweider 1969). This extinction coefficient approaches that of the most penetrating wavelength and can be determined experimentally with standard limnological techniques. Vollenweider (1965) discussed further the choice of the extinction coefficient for primary production studies. Futher implicit assumptions of this model are that the phytoplankton are homogeneously distributed in an isothermal euphotic zone. For a large lake such as Lake Michigan this is a reasonable assumption (Stoermer et al. 1967, Holland 1969).

Fitting the Model to Experimental Data

A method for estimating a, n, and I'_k from experimental data is needed to arrive at a practical working solution of the integral photosynthesis problem. For any assumed combination of the parameters, one may fit the model at the experimental irradiances. Some arbitrary function comparing the fitted model with the data points is needed and further a criterion for determining whether the fitted combination is in some sense the "best" possible fit. The most widely used of such functions is the expression

$$\chi^2 = \sum_{i=1}^{m} \frac{(O_i - E_i)^2}{E_i},$$

where O_i is an observed value of photosynthetic rage at a specified irradiance, E_i is the expected value at that irradiance under the assumed parameter combination, and m is the number of observations. In other words, this function is the summed squared deviances of the observed from the expected values, normalized by the expected values to make differences relative to the magnitude of the numbers being treated. Mood and Graybill (1963) proved that this widely used "goodness-of-fit" function is asymptotically distributed as the statistical chi-squared probability function.

With a given set of parameters, the computer can calculate χ^2. If it can also determine how χ^2 varies with a small change of each parameter, then a scheme can be devised to reduce χ^2. This general of formulation is obviously amenable to many numerical approaches. Indeed, Box (1966) has compared eight of the best modern techniques. He found that the best procedure is that of Fletcher et al. (1963). Their method is the one used in this work. The derivation of the partial derivatives of the χ^2 space needed for this technique are given by Fee (1971a), and Fee (1971b) presents the computer programs for estimating the model parameters.

Application of the Model to Lake Michigan

Methods

Samples were taken from the car ferry SS *Badger* operated by the Chesapeake and Ohio Railroad Company at five points on its cross-lake transit from Milwaukee, Wis., to Ludington, Mich. (Fig. 3). Sampling intervals ranged from 8–16 days. The vessel travels at 29 km/hr (18 mph) and makes the 150-km (93 miles) crossing (from dock to dock) in 5½ hr. The two stations designated as "inshore" were 4.8 km (3 miles) from the breakwalls of the two harbors; the three "offshore" stations were at the quarter and halfway points on the cross section. The two quarter point stations were about 29 km (18 miles) from the shores, while the center transect station was 48 km (30 miles) from the Wisconsin shore and 54 km (34 miles) from the Michigan shore. Station 1 (inshore Milwaukee) was normally sampled at about 0800 hr EST and station 5 (inshore Ludington) at around 1300 hr.

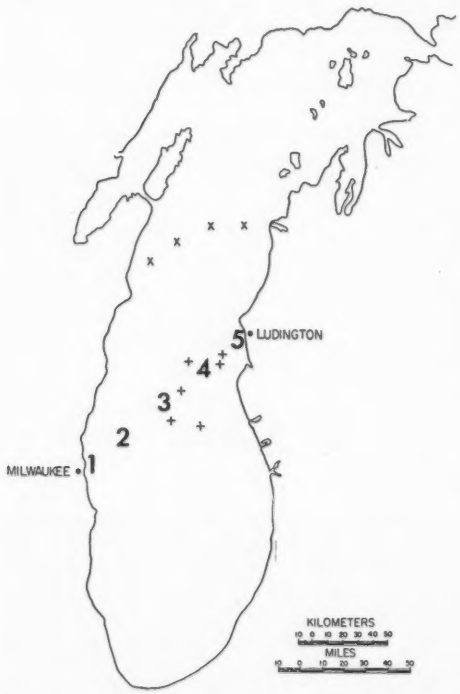

Fig. 3. Approximate positions of sampling stations in Lake Michigan (numerals), on car-ferry route. Plus signs, the stations of Parkos et al. (1969), and crosses, the stations of Schelske and Callender (1970).

Samples were delivered with a nonmetallic hose and faucet from a seachest whose intake was 4 m below the lake's surface. The extent of disturbance of the water column by the hull of the ship is unknown but was probably considerable. Thus, the samples may represent more homogeneous samples of the epilimnetic water than those obtainable through standard sampling techniques. Water exchanged virtually instantaneously between the seachest and the lake as evidenced by the sharp change in temperature frequently observed at the breakwalls of the two harbors. A 19-liter (5-gal) Pyrex carboy was filled in about 45 sec and all samples were extracted from this common source with a siphon, ensuring that the horizontal motion of the vessel would not bias the samples. Samples taken from this carboy were: nine light and one dark 150-ml bottles for ^{14}C uptake experiments; a 300-ml BOD bottle for the alkalinity titration; a 50-ml polyethylene bottle for determining *p*H.

Eight of the light bottles and the one dark bottle were immediately inoculated with ^{14}C, and after thorough shaking two light bottles were placed in each compartment of an incubator. The one dark bottle was placed in the compartment with the lowest light intensity. The dark bottles were prepared by wrapping bottles in black vinyl plastic electrical tape. All ^{14}C bottles were washed with concentrated hydrochloric acid between cruises. Blank bottles identical to the experimental ones were added and removed from the incubator as necessary to ensure that there was no change in light conditions as successive stations were processed.

Incubator — A light incubator (Fig. 4), in which bottles were held horizontally on vertically rotating discs at four different light intensities, was used to determine the rate of carbon uptake as a function of irradiance. The chambers were filled with drinking water from the ship. The bottles rotated five times per minute. Light was supplied by a bank of six 500-w T3Q tungsten–iodine vapor bulbs at one end of the incubator. Each bulb was backed with a paraboloid reflector of brightly buffed aluminum to direct the maximum amount of light toward the experimental chambers. Tungsten–iodine bulbs were chosen because they emit very high irradiances and have a long life with no change in the quantity of light emitted; the unidirectional arrangement allowed easy calibration. The amount of photosynthetically active light in the four chambers was determined with an ISCO model SR Spectroradiometer.

The measured irradiances (ly/min; integrals for spectrum between 4000 and 7000 Å) at various parts of the apparatus were:

Compartment 1	
front of bottle	0.1850
back of bottle	0.1463
reflection from back	0.0075
Compartment 2	
front of bottle	0.0548
back of bottle	0.0485
reflection from back	0.0026
Compartment 3	
front of bottle*	0.0162
front of bottle*	0.0167
back of bottle	0.0137
Compartment 4	
front of bottle	0.0084
back of bottle	0.0059

*Replicates.

The heat emitted by the light bulbs was largely removed by placing a rectangular Pyrex box directly in front of the first experimental chamber. This box was 5 cm (2 inches) wide and was filled with rapidly circulating cold water. By adding ice and/or hot water, the compartments were kept within 2 C of the temperature at the collection sites. All experiments were run in duplicate except for the dark bottles. Dark bottle ^{14}C uptake normally was very low; and separate experiments had shown it to be highly reproducible.

Radiocarbon — The rate of primary production was measured by the radiocarbon technique (Steeman-Nielsen 1952) as detailed by Strickland and Parsons (1968). Ten microcuries (μCi) of sodium bicarbonate solution ($NaH^{14}CO_3$) was injected with a 1-or 2-ml "insulin" syringe having a 5-cm needle into a 150-ml ground glass stoppered bottle from which the volume of the inoculum plus 1 ml had been previously withdrawn. The bottle tops were tightly secured with adhesive tape. After a thorough shaking, the bottles were incubated for 4 hr at which time the entire contents of the bottle were filtered on a 47-mm diam Millipore HA filter of pore size 0.45 μm. The filtration pressure was kept at between $\frac{1}{4}$ and $\frac{1}{3}$ atm. and the walls of the funnel were washed with distilled water after each filtration. Inoculations and filtrations were performed in a dimly lit room (less than 10 ft-candles of illumination). All samples were normally filtered within $\frac{1}{2}$ hr of the end of the incubation time and samples awaiting filtration

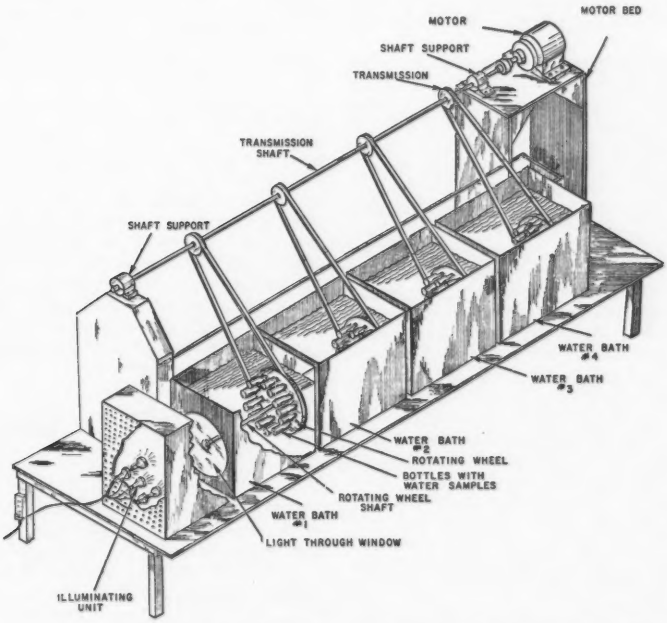

Fig. 4. Incubator used on car ferry for determining relation between irradiance and photosynthetic rate.

were kept in complete darkness. Figure 5 shows the light quality in the four chambers. While this spectrum differs from the in situ light quality of most waterbodies, Vollenweider (1960), Talling (1960), and Steemann-Nielsen and Willemcës (1971) reported that primary production is largely controlled by the total energy flux in the visible spectrum, regardless of quality. All optical surfaces were constructed of plexiglass and the sides of the experimental chambers were blackened to reduce reflection. The wet filters were immediately glued to 5-cm (2-inch) diameter aluminum planchets with rubber cement. After air drying they were placed in a desiccator containing silica gel and allowed to stand overnight. Subsequently the planchets were exposed for 20 min to fumes of concentrated HCl, to remove any inorganic radiocarbon, and were returned to the desiccator for redrying. The dried filters were counted for 10 min in a Nuclear Chicago model 1152 2-inch automatic, low background, gas flow, proportional counting system. The rate of carbon uptake is computed with the formula

$$\text{Photosynthetic rate} = \frac{(R_s - R_b) \times C \times 1.06}{H \times E \times A},$$

where R_s = the radioactivity (*cpm*) of the sample; R_b = the radioactivity (*cpm*) of the dark bottle; C = the total carbon (mg/m^3) available for uptake in the water; A = the radioactivity (*dpm*) added to the sample; E = the counting efficiency of the proportional counter for ^{14}C; and H = the number of hours the sample was incubated.

The factor 1.06 accounts for the slower rate of uptake of the tracer $^{14}CO_2$ relative to $^{12}CO_2$. To confirm that H could be varied at will to obtain convenient activities, an experiment was run in which replicates of a single sample were incubated for increasing time periods (Fig. 6). The uptake was still linear with time after a 4-hr incubation time. Added ^{14}C activities of 10 μCi were combined with this incubation time to obtain convenient counting rates. The efficiency of the proportional counter for ^{14}C counting was determined by running 10 replicates from a single experiment. Five of the samples were counted in the standard way on the planchet counter and the other five filters were counted by liquid scintillation, dissolving filters in AQUASOL (New England Nuclear Corp.). The degree of quenching was measured with an internal standard. The efficiency of the proportional counter was 19.2%. Dissolved inorganic carbon was determined by titrating a water sample with 0.1 N HCl to the bromcresol green-methyl red endpoint (ca. *p*H – 4.7). Phenophthalein alkalinities were analytically zero and thus mg ^{12}C/liter = 12× alkalinity in millimoles. The *p*H values (8.1–8.5) were measured electrometrically. Both alkalinity and *p*H were determined for each station but varied little seasonally or spatially; the titrations were performed within 6 hr of the time of collection, and the *p*H was measured within 2 hr of collection.

For the first 5 months of the study, $NaH^{14}CO_3$ supplied by the New England Nuclear Corporation was used: in 5-μCi/ml ampoules from May 24 through August 11 and in 10-μCi ampoules from August 21 through September 28. Post-inoculation (dark bottle) background activities with the latter ampoules were more than an order of magnitude higher than for the former. The mean for all the dark bottles for the five sampling dates before the 10-μCi ampoules were used and the first four dates on which another method was used was 38.7 cpm (95% confidence interval 34.5–43.0), in comparison with 730.6 cpm (606.7–854.5) for the three dates on which the 10-μCi ampoules were used. Investigation indicated particulate contamination of the ampoules, as Platt and Irwin (1968) reported concerning ampoules from this company.

After September 28 the following procedure was used. Dry crystalline $NaH^{14}CO_3$ was purchased in 1-μCi quantities. This was mixed with 100 ml of glass-distilled water previously adjusted to a *p*H of 9.5 with NaOH. All glassware and the water were autoclaved immediately before mixing the radiocarbon stock solution. The solution was stored in ground glass stoppered bottles wrapped in aluminum foil and was kept refrigerated. The ground glass joint was smeared with stopcock grease to prevent evaporation. No loss in activity over a 2-week period of storage was detected. Not only were dark bottle counts reduced to the previous acceptable levels but the variation of replicates decreased to the lowest observed values.

The stock solutions thus prepared were standardized by a liquid scintillation technique. A scintillation solution composed of 12 ml of phenyl-oxazole-phenyl (4 g/liter) (POP) plus phenyl-oxazole-phenyl-oxazole-phenyl (0.05 g/liter) (POPOP) in a toluene solution with 2.8 ml of absolute ethanol and 0.1 ml of monethylamine formed the base for scintillation counting in a Nuclear Chicago Mark I liquid scintillation counter. To this solution was added 0.1 ml of a radioactive ^{14}C solution made by mixing 1.0 ml of the stock solution being standardized with 49.0 ml of glass-distilled water previously adjusted to a *p*H of 9.5 with NaOH. This gave data in the range of 30,000 to 40,000 cpm, which is high enough for statistical accuracy but low enough to avoid coincidence errors. Two methods of determining the counting efficiency of the Nuclear Chicago Mark I system with this solution were used. An automatic external standard (AES), built into the machine, was counted on a blank composed of the above solution mixed with 0.1 ml of distilled water and on a ^{14}C standard. The efficiency (E) was computed from

$$E_{AES} = \frac{\text{AES of blank}}{\text{AES of } ^{14}\text{C std}} \times \text{efficiency of counting } ^{14}\text{C std.}$$

An internal standard method was used as an independent check of the ^{14}C counting efficiency of the liquid scintillation counter by mixing the scintillation solution described above with 0.1 ml of a toluene-^{14}C standard (IS). The efficiency was computed from

$$E_{IS} = \frac{\text{Int. std} - \text{blank}}{\text{Absolute activity of } ^{14}\text{C std}}.$$

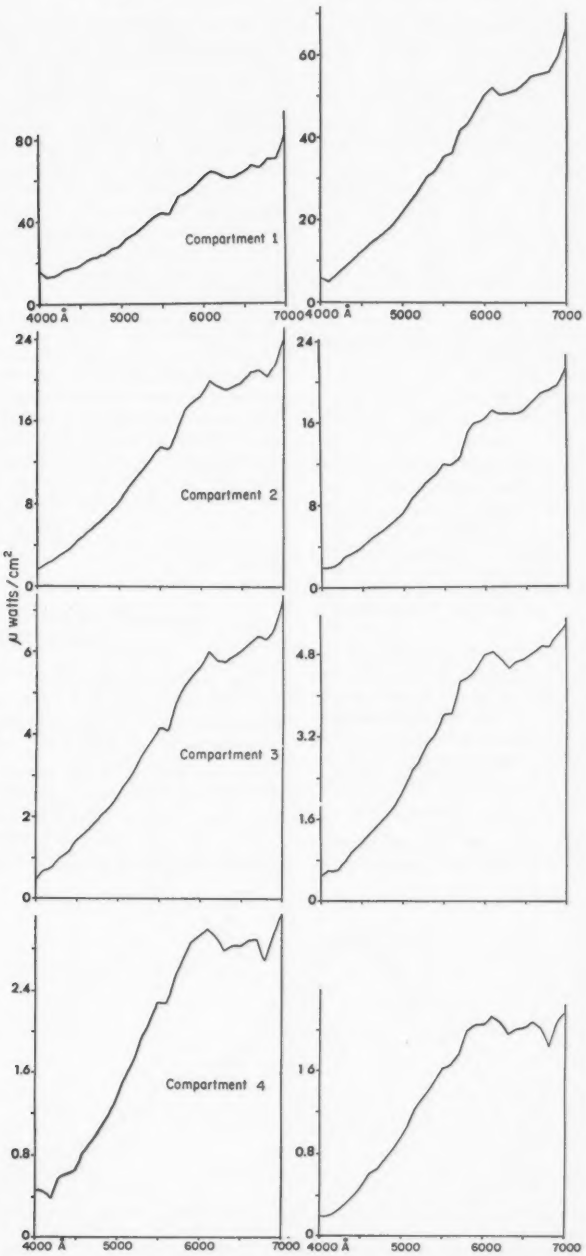

FIG. 5. Light climate of incubator. Left, in front of bottles; right, back of them.

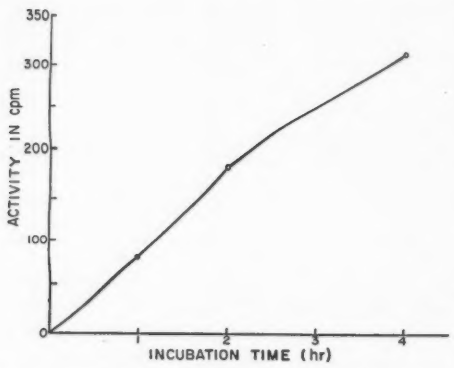

FIG. 6. Effect of incubation time on photosynthesis as measured in incubator at station 4, June 26, 1970.

The two methods usually resulted in efficiencies of about 84% and only rarely differed from each other by more than 2%. The internal standard method was slightly more variable than the other, probably as a result of errors in pipetting the toluene-^{14}C standard solution. The high volatility of the toluene standard could also contribute to this small error. Stock solutions were standardized within 1 day of the day the experiments were performed.

Temperature — The water temperatures of the cooling water intake of the car ferry were monitored continuously using a Taylor FULSCOPE circular chart recorder having a 12-inch diameter chart calibrated over the range 0–100 C and making one revolution in 12 hr. A mercury thermometer was used to determine temperatures of the samples.

Light — The published values of 0.2 m^{-1} (Beeton 1962) and 0.37 m^{-1} (Norden 1968) were used for the extinction coefficients of the offshore and inshore Wisconsin waters, respectively. Difficulties of instrumentation prevented direct measurements of this parameter in this study, but it is probably a stable parameter for offshore Lake Michigan. Since, as it will be shown, the inshore Michigan station was similar in all other biological and chemical properties to the offshore stations it was assumed to have the same extinction coefficient.

Total incident solar irradiance was measured with a Kipp and Zonen CM-3 Solarimeter equipped with a Moll thermopile (14 elements of constantan–manganin) mounted on a five-storey building within ½ mile of the lakeshore at Milwaukee, Wis. A Honeywell strip chart recorder monitored the output of the solarimeter continuously. A d-Mac Mechanical Digitizer was used to convert these data to 80 column punched cards for computer analysis. These digitized records were converted to surface irradiances equally spaced in time using the computer program documented by Fee (1971b). The solarimeter calibration supplied by the manufacturer was checked with an ISCO model SR Spectroradiometer equipped with a model SRR Programmed Scanning Recorder to obtain light energy in the visible part of the spectrum. Since the light source of the incubator on the car ferry was calibrated with the same instrument, any error in the calibrations of these two devices would cancel out.

Seasonal Variation of Physical Parameters

The surface temperatures at the two inshore stations were higher in the spring but not consistently lower in the autumn than those at the three offshore stations (Fig. 7). Futher, probably as a consequence of the prevailing westerly winds, the inshore Michigan (eastern) station was warmer than the inshore Wisconsin station at all times. Station 4, the offshore Michigan station, was always the coldest station during the heating phase,

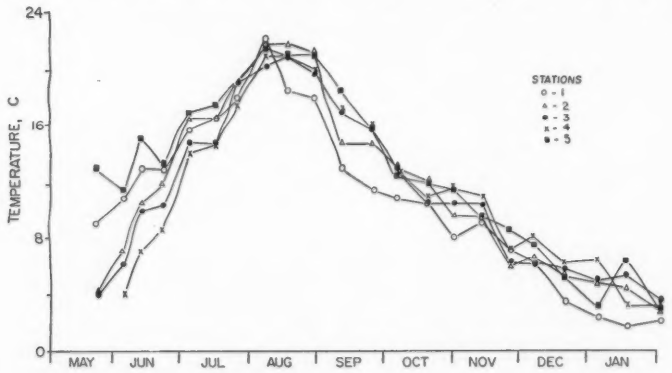

FIG. 7. Surface water temperatures at sampling stations, May 27, 1970, February 1971.

while station 1 was the coldest station during the cooling of the lake. The highest temperatures were observed in mid-August. The lake cooled less rapidly than it heated. Large horizontal temperature gradients were more evident in the spring than in the fall as a result of this seasonal difference in the rate of heat exchange. The lake was never horizontally isothermal.

Though the total daily surface irradiance (Fig. 8) varied greatly, the seasonal trend was smooth.

Seasonal Variation of the Model Parameters

P_{opt} (Fig. 1), (Talling 1957b) is defined as the rate of assimilation of carbon per unit volume and time, i.e. mgC/m^3 per hr, at the irradiance best suited for photosynthesis. In the model it is used as a physiological measure of biomass and was experimentally determined by exposing identical samples to a series of irradiances in the incubator. The highest observed rate of carbon uptake was operationally defined as P_{opt} regardless of where it occurred in the incubator.

P_{opt} was higher at station 1 than at the other stations (Fig. 9) — usually by at least a factor of two. Futher, the seasonal pattern of P_{opt} at station 1 was different from that seen elsewhere. At station 1 there were two periods of maximum P_{opt}, in late June and early October; at the offshore stations and station 5, there was one very broad and smooth peak, in September and October. Moreover, the pattern at station 1 was erratic, both peaks being based on single points. It is unlikely that the sampling interval of 2 weeks was sufficiently short to resolve the actual changes at station 1.

To fit Vollenweider's equation to the experimental data, it was necessary to determine whether the pronounced numerical differences between station 1 and the other stations, evident in the other parameters already considered, were also present in the curves relating relative carbon uptake to irradiance. This was accomplished by following variations in P/P_{opt} at four light intensities (Fig. 10). Seasonal changes at all stations were similar. In particular, station 1, which was so different in the magnitude of carbon uptake, fell in the center of the scatter of points showing relative carbon uptake. The optimum rate of carbon uptake, i.e. $P/P_{opt} = 1.0$, occurred in compartments 1 or 2 with little or no seasonal pattern.

During the summer P/P_{opt} in compartment 4 decreased to 0.05 while the winter values at the same irradiance increased to about 0.50. Another sharp decrease occurred in early October. Thus, the algae were making much more efficient use of low irradiances in the winter that in the the summer. It will be shown that these changes of P/P_{opt} at low irradiances are of direct importance in determining the seasonal variations in integral daily production. For the present, it is only important to note that the trend of P/P_{opt} was identical at all stations.

Because of the similarity of the P/P_{opt} data at all stations, all the results from a given date were pooled in fitting the model. This gives more reliable statistics since random errors at any single station are made less important. Using the computer program documented by Fee (1971b), the parameters a, n, and I'_k were computed from the data in Fig. 10. Table 1 gives the numerical output and

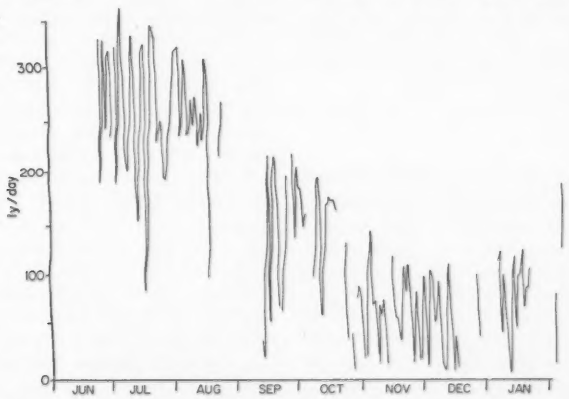

FIG. 8. Total surface irradiance measured at a point within 0.5 miles of the lakeshore at Milwaukee, June 26, 1970– Feb. 3, 1971; days on which instrument was inoperative appear as gaps. Data corrected to give only solar energy in visible portion of spectrum.

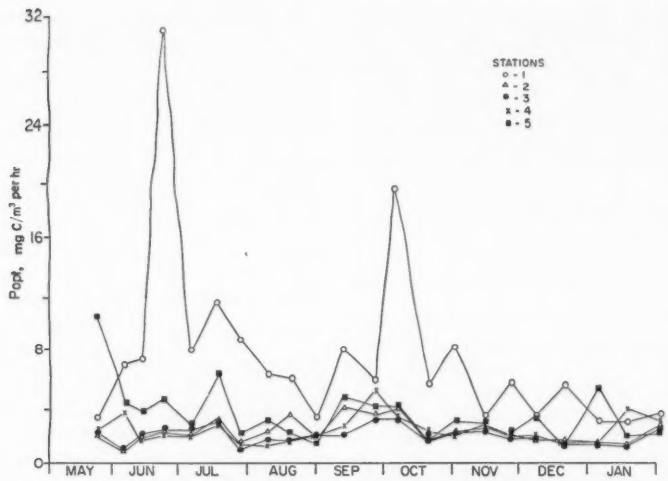

FIG. 9. P_{opt} at sampling stations during study period.

Fig. 11 shows typical experimental data together with the fitted model for midsummer and late autumn. Complete data for this period are presented by Fee (1971a).

The parameter I'_k is inversely related to the efficiency of carbon uptake at lower light intensities. As shown in Fig. 12, it was highest in midsummer, achieving the maximum observed value of 0.056 cal/cm² per min in late July; the winter values were nearly constant. The parameter a almost invariably assumed the value of unity, while n varied like I'_k but was more erratic (Fig. 12). Thus the model quantitatively shows that the phytoplankton exhibits greater photoinhibition and less efficient use of light in the suboptimum range of intensities in summer than in winter.

Model Output

There was great variability in daily integral productivity at each station (Fig. 13). Rodhe et al. (1958) noted similar variability using in situ measurements of carbon uptake in Lake Erken (Sweden) and ascribed the variability to changing biomass distribution due to wind. Based on this interpretation, they predicted that daily variability would be less on very large than on small bodies of water. My results show that this is not so; the sort of daily variability they observed can be explained solely on the basis of variations in isolation.

During midsummer — a time of high surface irradiance — there was a sharp decrease in the integral carbon uptake at all stations. As shown in Fig. 14, the decrease was much greater than can be accounted for by the decrease in P_{opt}, the added effect being due to the decreased efficiency of carbon uptake at suboptimum irradiances.

The lowest integral productivities were observed from July 26 to August 5, which were the dates which had the highest values of n and I'_k. Note, for example, that August 1–3 had surface irradiances as high as July 16–17 but the system output was only 76% as high even after the effect of P_{opt} was removed from the model. This may explain why attempts to correlate surface irradiance and integral photosynthesis (Edmondson 1956) have been invariably frustrated. Correlations which hold for short periods have been found inadequate for other times. To my knowledge, this is the first time that such physiological variables have been shown to be of direct importance in determining integral primary production.

Station 1 showed the highest daily rates of integral production, usually slightly more than double those at the offshore stations on the same dates from early summer through autumn. The values at station 5 were between those at station 1 and the offshore stations during this period. The maximum autumn values were slightly higher than the spring values except at the midlake stations, where they were nearly identical. Station 1 showed a very high peak (>3000 mgC/m² per day) at the start of the study period, primarily because of a single high P_{opt} value at that time. All stations had the lowest observed values in early winter, although the numerical differences between this period and

midsummer were small. On some midsummer dates and on about half of the winter dates the offshore values exceeded those of the inshore Wisconsin waters. Because of the different extinction coefficients used, this happened whenever P_{opt} at station 1 was less than 1.85 times P_{opt} at the offshore stations. In fact, the values on a given date are identical except for the factor P_{opt}/ε and the numerical integrations for all stations need be made only once, the output being multiplied by P_{opt}/ε for each station to give the final answer. This results in a great saving in computer time. Ideally, one

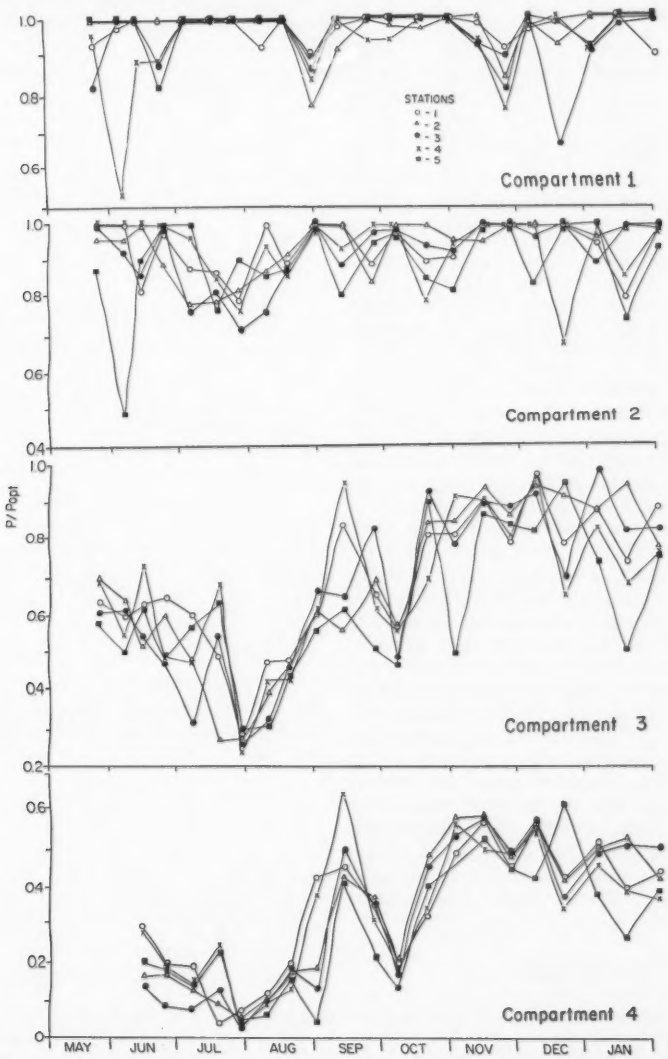

FIG. 10. Relative production rates (P/P_{opt}) for samples from the five stations in the four compartments (1, highest irradiance; 4, lowest) of car-ferry incubator.

TABLE 1. Parameter values a, n, and I_k' and goodness of fit function (χ^2) computed from the car-ferry incubator experiments.

Date	a	n	I_k', ly/min	χ^2
June 16 1970	1.000	0.081	0.024	0.302
June 26	1.000	0.254	0.028	0.302
July 8	0.958	0.069	0.031	0.396
July 20	0.248	0.000	0.028	0.624
July 30	1.000	0.531	0.056	0.316
Aug. 11	1.000	0.268	0.041	0.290
Aug. 21	1.000	0.078	0.033	0.075
Sept. 2	1.000	0.286	0.022	0.468
Sept. 14	0.672	0.000	0.013	0.267
Sept. 28	1.000	0.034	0.019	0.192
Oct. 8	1.000	0.156	0.028	0.176
Oct. 22	1.000	0.047	0.014	0.264
Nov. 3	0.618	0.000	0.010	0.036
Nov. 16	1.000	0.074	0.010	0.043
Nov. 28	1.000	0.181	0.013	0.041
Dec. 9	1.000	0.056	0.010	0.134
Dec. 22	1.000	0.117	0.014	0.298
Jan. 6 1971	0.917	0.093	0.012	0.131
Jan. 19 1971	0.551	0.227	0.012	1.287
Feb. 3 1971	1.000	0.068	0.014	0.085

should have records of surface irradiance at each station and then the integrations must be performed separately for each station.

The average production rates for the study period were 670, 380, 331, 381, and 512 mgC/m^2 per day for stations 1 through 5, respectively. This time period probably includes the extremes likely to be encountered — at least at the offshore stations — and the averages are thus probably reasonably close to the true averages for the year. Using this assumption, the estimated annual rates of carbon fixation are 247, 139, 121, 139, and 187 g C/m^2 per year, at the five stations, respectively. These are the first estimates of annual production for Lake Michigan, the values for the offshore stations probably being much closer to the true values than are those for the other two because of reasons already given. The similarity of stations 2–4 is striking and is strong evidence of the horizontal homogeneity of the model parameters in the offshore waters of Lake Michigan.

Test of Model

To test the veracity of the model output, an in situ station 10 miles due eats of Milwaukee, Wis., was occupied on October 19 and November 20, 1970 using the research vessel *Neeskay* of the Center for Great Lakes Studies. Samples were taken at five depths with a van Dorn water bottle. Using experimental techniques identical to those previously described for the car-ferry work, samples prepared for measuring radiocarbon uptake were suspended at the depths of collection for 4 hr. Temperature and plant pigments were also sampled to test the assumption of a vertically homogeneous algal distribution. The choice of sampling dates, though dictated by ship availability, was fortunate: the 4-hr incubation time, which began at midday,

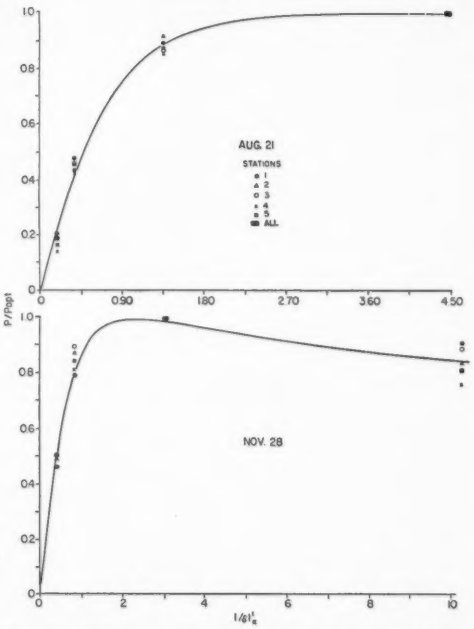

FIG. 11. Experimental values of P/P_{opt} vs. I/I_k' for the five stations, and the fitted curves (equation 1) for two dates in 1970.

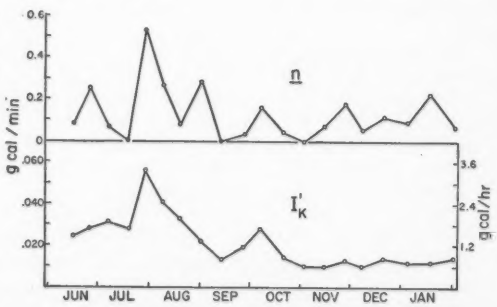

FIG. 12. The model parameters n and I_k' calculated from the pooled incubator data, June–February.

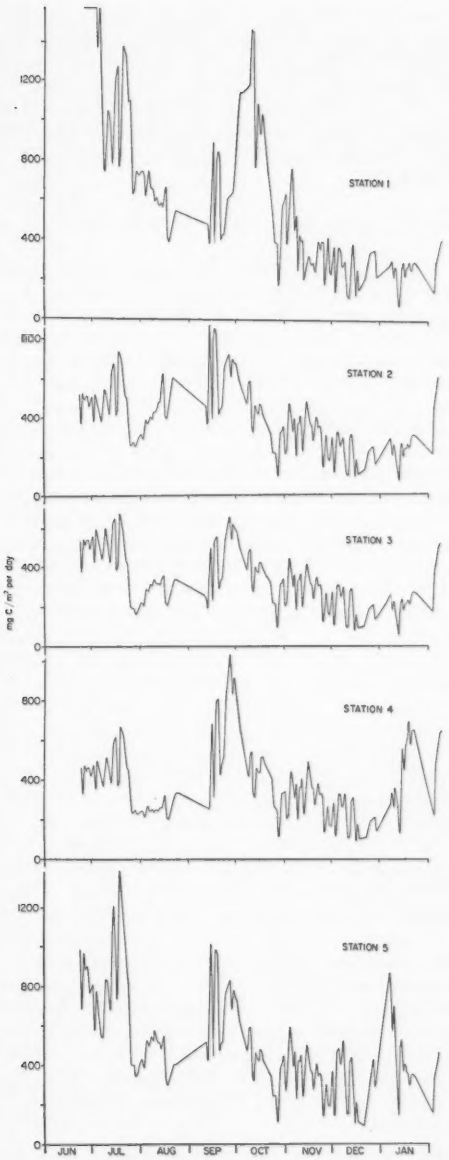

FIG. 13. Computed daily integral primary production at the five stations, June–February. The data are plotted as unbroken curves even though the gaps in the light data (Fig. 8) produce corresponding gaps in the model outputs. P_{opt} values for dates between sampling runs were obtained by linear interpolation, and the values of a, n, and I'_k from a given run were applied uniformly to all dates between the midpoints of the intervals between the run and those immediately before and after. Dates on which the solarimeter was inoperative are omitted since there is no adequate method of predicting the detailed nature of surface irradiance. The extinction coefficients were assumed to be invariant over time.

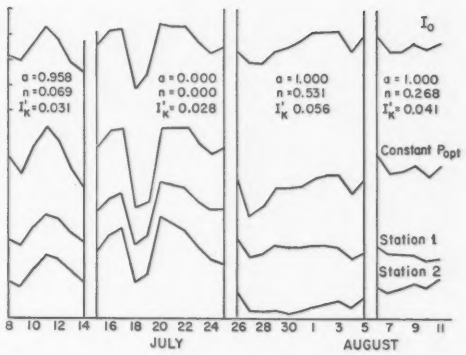

FIG. 14. Detailed analysis of integral primary production from July 8 through August 11, 1970 for two representative stations. Uppermost curve, total daily surface irradiance recorded at Milwaukee; second, model output using a constant value of P_{opt} for entire period and thus showing effect of variation of other model parameters; third and fourth, model outputs for stations 1 and 2, including the effect of P_{opt}.

was about half of the daylength on these dates so that the daily integral rates required for comparison with the model output could be estimated by simply doubling the experimental rates.

On both dates the data for log (P/P_{opt}) vs. depth were almost linear below optimum depth, indicating vertical homogeneity, and the slopes of the curves were 0.15 and 0.39, respectively. These are very close to the values of 0.20 and 0.37 for the inshore Wisconsin and offshore waters, respectively, which were derived from the literature and used in the model computations. This indicates that different water masses were present at the station on these dates and, therefore, the in situ rates are compared with those at station 2 on October 19 and at station 1 on November 20. The figures also show that the vertical distribution of plant pigments was practically uniform on both dates, which corroborates the conclusions just drawn from considering the semilogarithmic plots. Water temperatures on October 19 were 10 C from 0 to 30 m and on November 20 it was 9.5 C down to 27 m and 8.8 C at 30 m.

On October 19 the model predicted 399 mg C/m^2 per day while the in situ value was 419. On November 20 the model output for station 1 was 232 compared to 199 mg C/m^2 per day in situ. As these are entirely independent estimates, the agreement is most encouraging. By making use of more information, some of the discrepancy between model predictions and in situ measurements can be further explained. On November 20 the surface light within the incubation period was 64% of the daily total. Assuming simple proportionality of the daily rate to surface irradiance gives a corrected in situ value of 156 mg C/m^2 per day. Comparing the values of P_{opt} used in the computations allows correction of the model output for the differences in the algal densities at station 1 and the in situ station. The P_{opt} used for the model calculation was 4.28 mg C/m^3 per hr while the in situ rate was 2.99. It is reasonable that the P_{opt} values for station 1 would be high compared to those at the in situ station since the former is only 3 miles offshore compared to 10 for the latter. In fact, on both dates the in situ P_{opt} values were greater than those at station 2 and less than at station 1. Using the in situ parameter for the model calculations gives 162 mg C/m^2 per day, which differs from the corrected in situ experimental output by only 6 mg C/m^2 per day (3.8%). On October 19 the surface irradiance during the experimental exposure was 49.8% of the total daily irradiance and the difference between the in situ and model output values was 20 mg C/m^2 per day (4.9%).

While it is obvious that only two experiments are inadequate to corroborate all of the model computations, the degree of correlation achieved is high. Certainly the comparisons show that the model output is of the correct order of magnitude. It is of much greater use for predicting the actual variations of primary production than the usually employed in situ method. For example, the model output for station 1 on November 19 and 21, the days preceding and following one in situ experiment, were 283 and 311 mg C/m^2 per day compared to the November 20 value of 232. It is obvious that even weekly in situ measurements — which would be prohibitively expensive and tedious on Lake Michigan — would give a poor indication of the variability in the system.

Discussion

The model results at the inshore Michigan station agree well with the range of values reported by Saunders et al. (1962), who measured in situ integral production in Grand Traverse Bay, Mich., during the summer of 1959. As far as I know, these are the only available data for Lake Michigan directly comparable to the model output. The present results thus represent the first estimates of integral production of the offshore waters of Lake Michigan. These are also the most extensive data on primary production for any of the Great Lakes.

Physiological Parameters of the Model

The only physiological parameter of the model previously measured on Lake Michigan is P_{opt}. Schelske and Callender (1970) reported offshore productivity rates August 1969 cruises in Lake Michigan at four stations (Fig. 3) north of the car-ferry transect. Their average value was 3.72 mg C/m^3 per hr, in comparison with the average value 2.32 found in the car-ferry incubator during the same dates 1 year after their study. Parkos et al. (1969) found an average value for four offshore stations close to the car-ferry transect (Fig. 3) of 1.69-mg C/m^3 per hr during mid-July of 1967. This value falls between the average offshore productivities observed on July 20 and 30 during 1970. Their data for a similar set of stations from October 6 to 8 yielded an average value of 5.58 mg C/m^3 per hr, which compares with an average of 3.82 mg C/m^3 per hr observed at the offshore car-ferry stations on October 8, 1970.

Direct comparisons with these data are not as straightforward as might be supposed, since the conditions for the measurement of P_{opt} were not standardized. For example, high rates could result from incubating the samples at temperatures above the in situ temperature (Talling 1957b). Parkos et al. (1969) made no attempt to simulate in situ temperatures while Schelske and Callender (1970) used surface water to cool the incubating bottles. Further, in the present work, it was shown that the optimum irradiance for photosynthesis was by no means constant. It occurred in either compartment 1 or 2 of the incubator except on one occasion, when it was found in compartment 3. Neither of the previous studies used a range of known light intensities. Schelske and Callender (1970) used sunlight — which varies greatly from day to day; Parkos et al. (1969) used a single fixed irradiance.

On the basis of the model results, it does not appear likely that the P_{opt} values obtained from the car ferry could be as much as 40% low, as the data of Schelske and Callender (1970) imply. If the model output is adjusted upward by this amount, the integral production rates reach clearly unreasonable levels.

An important result, not anticipated, was that the model parameter a was almost invariably unity. The parameter a was not equal to 1.0 on only three dates; and on two of these instances it was close to 1.0 (0.96 and 0.92), while the third case was statistically the worst fit of all the data sets (Table 1). Note that by inspection of the model (equation [1]) it can be seen that when n is zero the value of a is arbitrary and can thus be assumed to be unity. The parameter n is thus shown to be solely responsible for modelling light inhibition in Lake Michigan, and Vollenweider's equation can be simplified accordingly to

$$\frac{P}{P_{opt}} = \frac{I/I'_k}{[1 + (I/\delta I'_k)^2]^{(n+1)/2}},$$

$$\text{where } \delta = \sqrt{n}\ (1+1/n)^{(n+1)/2}.$$

The general applicability of this new and simpler equation — derived here through purely empirical means from Vollenweider's more general equation — remains to be determined, since this study is the first that has measured a and n from experimental results. If this result proves general, then the problem of fitting the model will be much simplified. A straightforward binary search technique, such as that presented by Fee (1968), could be readily adapted for this purpose. That the numerical model results are not an artifact of the computer program was demonstrated by feeding the computer manufactured data; the output from the program always converged to the appropriate parameter combination. The fact that on three occasions the value of a was other than unity is further evidence that the program was functioning properly. Certainly, the computer fits (Fig. 12), are reasonable. Indeed, the least acceptable fit is associated with the only date on which a was not equal to unity (Table 1).

A point which appears from inspection of the computer fits is that the model consistently overestimates the relative productivity at the lowest irradiance and underestimates it at the next higher irradiance. This is caused by forcing the model to pass through the origin of the coordinate system and could be remedied by adopting the following modification

$$\frac{P}{P_{opt}} = P_o + \frac{I/I'_k}{[1 + (I/I'_k)^2]^{(n+1)/2}},$$

where P_o, the intercept on the P/P_{opt} axis, represents respiration. However, the results of the present computations are sufficiently accurate for the purposes of this modeling effort. Certainly, the deviations from the model — though consistent — are minor quantitatively.

Use of linear interpolation to determine P_{opt} on the dates between sampling runs is undoubtedly valid for the offshore stations where temporal changes in all parameters were gradual; its validity in the inshore waters — particularly at station 1 — is questionable. It is obvious that some method of monitoring P_{opt} in greater detail at station 1 is needed before much dependence can be placed in the model output for this station. It would be very tedious and expensive to perform the incubator experiments on a daily basis; for short intervals this is obviously the best method. For routine and extended surveys, the close correlations found between P_{opt} and the plant pigments at station 1 (Fee 1971a), together with continuous monitoring of in situ chlorophyll a with a fluorometer (Lorenzen 1966), may provide the tools needed.

One of the most important — and previously unsuspected — phenomena discovered was the seasonal trend of relative photosynthesis (P/P_{opt}) at low irradiances. The values of I'_k, which is a quantitative measure of this trend, were in good agreement with the range reported in the literature (Talling 1957b, Vollenweider 1960). The midsummer decrease of efficiency was shown to be significant in causing the midsummer decrease (Fig. 14) of integral production. Figure 2 makes it clear why this parameter is of such importance in calculations of integral production — due to the exponential decline of light, most of the depths have irradiances which are at suboptimum irradiances for photosynthesis.

Uses of the Model in Lake Typology

The model output can be used in various ways. For computing the effect of the nutrient loading into a water body, the total quantity of carbon fixed by the system must be known; and therefore estimates of vertically integrated primary production rates are required. Moreover, in the calculation of production efficiencies at higher trophic levels (e.g. zooplankton and fish) only the integral rates are of any use. Thus, the integral rates supplied by the model provide the key to linking the phytoplankton to the functioning of the other parts of the ecosystem. Other uses of these rates have been proposed by investigators concerned with water quality (Rodhe 1958).

Inevitably then, the question of the trophic classification of waters arises. Based on theoretical grounds, production rates would seem to provide the best of all criteria for lake typology since the

rate of carbon fixation is an integrator of the nutrient and physical factors affecting a lake. For example, a lake which had a high nutrient load but received little light could still be unproductive because the algae would be physically limited. On the other hand, if a toxic chemical were present, very high nutrient loads and light levels would still not produce a eutrophic lake. If we decide that primary production is potentially useful for classifying lakes according to trophic status, which measure of production should be used? The total rate of carbon fixation per unit area would seem a logical unit since this is the unit available to the other trophic levels to support their biomass, and the accumulation of excess algal biomass is the most obvious evidence of a eutrophic condition. However, it has become apparent that integral productivity is a poor parameter for this purpose because of the inverse relation between transparency and primary production. As primary production per unit volume increases there is normally an increase in biomass with a concomitant drop in transparency. The net result of this is that the euphotic zone becomes shallow in a highly productive lake. Some numerical examples will demonstrate the magnitude of this phenomenon. Ryther (1960) showed that the integral production of the Sargasso Sea, which is one of the most oligotrophic areas in the oceans, was nearly equal to that of a highly eutrophic pond in South Dakota, though the optimum rates of production per unit volume differed by a factor of 150 and the standing crops of phytoplankton by at least a factor of 450. Concerning the Great Lakes, Saunders et al. (1962) found that integral production of Grand Traverse Bay in northern Lake Michigan was three times as great as in Douglas Lake, Cheboygan County, Mich.; Douglas Lake is a shallow, naturally eutrophic lake. In this case, all the measurements were made with the same techniques during the same summer and the water bodies are close enough together so that insolation could be considered identical. Indeed, using the classification based on integral primary production rates proposed by Rodhe (1969), the results of the present study indicate that the offshore stations of Lake Michigan are eutrophic. Any reasonable interpretation of the present conditions (Beeton 1965, 1969) goes against this conclusion. Clearly, integral production rates alone are of questionable value in classifying lakes. Some account must be taken of both the optical conditions of the water body and the optimum volumetric rate of carbon uptake.

To supply such a trophic criterion, Vollenweider (1960) proposed that the ratio of the daily integral rate to the optimum volumetric daily rate of primary production ($\Sigma\Sigma\ P/\lambda P_{opt}$, in the notation of this work) be used for classifying lakes. From the general solution of the photosynthesis integral (equation [2]), this ratio is

$$\frac{\Sigma\Sigma P}{\lambda P_{opt}} = \frac{1}{\epsilon}\frac{\delta}{2}\int_{-1}^{1}\int_{0}^{I_o(x)/\delta I'_k} \frac{dy}{[(1+y^2)\,(1+(ay)^2)^n]^{1/2}}\,dx \qquad [3]$$

and has the units of meters. Note that P_{opt} must be multiplied by λ so that the two rates refer to the same time interval. According to Vollenweider's theory and preliminary results, this expression will decrease as lakes become more eutrophic.

Figure 16 presents Vollenweider's function calculated from the model output. Equation [3] makes it clear why this coefficient is the same for stations 2 through 5, i.e. they all were assumed to have the same extinction coefficients. The parameters a, n, and I'_k were calculated once for all stations and $I_o(x)$ was assumed to be the same for all stations. Thus, the only variable on the right side of the equation is ϵ.

It is immediately obvious from Fig. 16 that there is no seasonal trend to this ratio except for a decrease in late July and August. The average value for station 1 is 6.21 m and for all the others it is 11.49 m. The variability is very high, which is to be expected since equation [3] shows that this parameter is a function of ambient irradiance. Therefore, more significant than the average value is the maximum value, which Fig. 16 shows is constant at about 16 m for stations 2 through 5, and 8.4 m at station 1. These values seem to represent the extremes beyond which the system is incapable of going and as such are of more value for comparative purposes than averages, since averages will be decreased by cloudiness more in some areas than in others.

The difficulties of comparison with other available data are also made obvious by Fig. 16. A few in situ measurements of the ratio, which is all that are available from other studies, would almost certainly miss the peaks. Even more important perhaps is the fact that isolated observations would probably miss the lack of trend because of the high variability. The peak output of the system, which seems to be of the most significance for comparison of water bodies, can almost certainly not be obtained with standard in situ techniques.

Such as they are, the other available data (summarized by Ahlgren 1970) indicate that the offshore

stations of Lake Michigan are as oligotrophic as any lakes for which the ratio has been calculated. This is true whether the mean or maximum values are used. In Ahlgren's list, the only other lake that approaches these offshore stations is Lake Victoria in Africa. The value at station 1 also lies at the upper end of the spectrum of the available data but is less than the Lake Victoria value (11 m). I have calculated from the data of Ryther (1960) that the Sargasso Sea, an ultra-oligotrophic system has a value of 52 m. Firm conclusions regarding these comparisons must be avoided until more and better data are available from other systems, but it is clear that this is a better scheme than that based solely upon daily integral rates.

Need for Futher Knowledge of Variations of the Optical Properties of the Water

We need to know much more about light penetration in the Great Lakes. The difference between the photosynthetic ratios (equation [3]) calculated for station 1 and the others is simply due to a different choice of the extinction coefficients. There are only a handful of values of this parameter available for all of the Great Lakes, and we know nothing about its seasonal variability. This represents the principal deficiency of this study. The model is particularly sensitive to values of this parameter since it is reasonably small and occurs in the denominator (equation [2]). For example, a change of ε from 0.2 to 0.3 decreases production by 33%. In the present study the magnitude of the differences in integral production between station 1 and the others was nearly halved because of the choices of ε. The variable turbidity due to wave scouring in the inshore areas is probably more stochastic than seasonal. The assumed invariance of ε in inshore waters undoubtedly masks a great deal of actual variation from this source. On the other hand, the phytoplankton are never dense enough to color the offshore waters of Lake Michigan, and variability of the extinction coefficient there is probably small. However, until some measurements are made, these remain unverified assumptions.

Concerning the measurement of ε in the Great Lakes, Fig. 15 is particularly interesting. The linearity of P/P_{opt} on a semi-logarithmic plot and the closeness of the slopes of these lines to the asssumed values of the extinction coefficients is striking. Technical difficulties have been largely responsible for lack of knowledge of the vertical extinction of light in the Great Lakes. Large boats, which are necessary for work on these water bodies, tend to shade instruments and rough water conditions make it difficult to achieve reproducible measurements. Moreover, even with the values so obtained, these is some ambiguity as to which extinction coefficient to use (Vollenweider 1965), since each wavelength is extinguished differently.

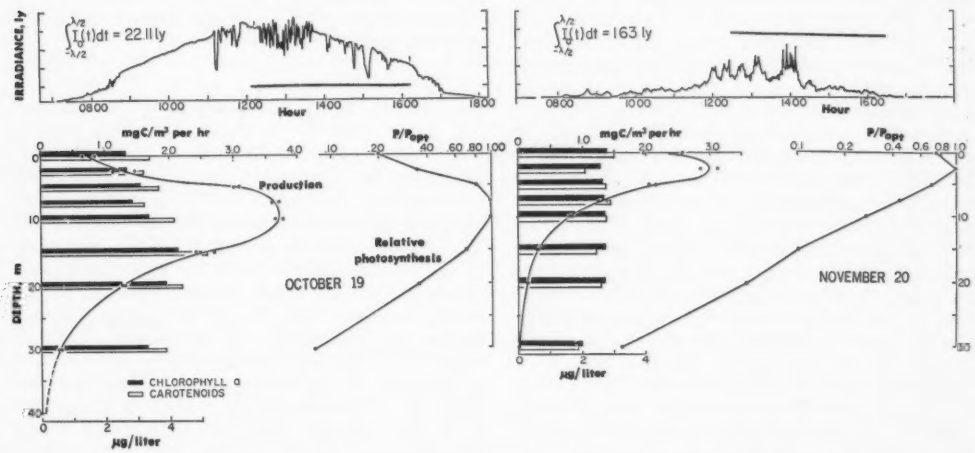

FIG. 15. Primary production and plant pigment concentrations in relation to depth for 4-hr (solid bar) incubations in situ 10 miles east of Milwaukee on two dates in 1970, and relative photosynthesis. Irradiance values, taken within 0.5 miles of the lakeshore, are for visible light at surface, the totals for before, during, and after incubation being 73.8, 80.9, and 7.8 ly on October 19 and 7.8, 14.1, and 0.2 on November 20. The dashed part of the production curve is the exponential extrapolation of the curve above 30 m and was required to measure the depth integral.

Figure 15 shows that we can use the in situ carbon uptake experiment to obtain an integrated value for total energy available for photosynthesis at a given depth, which is the parameter we are after. The phytoplankton are being used as an integrator of total available light.

In addition to Fig. 15, the data of Saunders et al. (1962) can be used as a preliminary test of this reasoning. They performed two kinds of experiments: (1) a surface sample was suspended at all sample depths (0, 5, 10, 15, 20, and 25 m); the variations in uptake should be due to decreasing light alone, and this series of experiments they referred to as the "transparency series"; (2) samples from each depth were resuspended at the same depth; this series they called the "in situ" series. By plotting P/P_{opt} on semilogarithmic paper against depth in the manner shown in Fig. 2, I have calculated the slopes of the lines, the values being as follows:

	July			August				
Date	24	27	29	6	11	13	16	20
Transparency series	0.19	0.18	0.21	0.28	0.28	0.34	0.31	0.42
In situ series	0.21	0.20	0.30	0.35	0.30	0.37	0.33	0.41

The data were uniformly linear on these semilogarithmic plots. They reported extinction coefficients measured with a photocell that varied from 0.16 to 0.39, which is in remarkably good agreement with the extinction coefficients I have calculated from their ^{14}C uptake data. This variability is much greater than that likely to occur in the open waters of Lake Michigan since it is an isolated bay. Indeed, Lauff (1957) reported that Secchi disc depths at any station in Grand Traverse Bay can vary by a factor of two to three on any given day. The values given by the two types of experiments are in very good agreement except for two dates. These deviations could be caused by either vertical inhomogeneity of the phytoplankton or the effect of temperature on carbon uptake. Either of these is likely since the thermocline was only 5 m deep. Certainly, the agreement is good enough to warrant further investigation of the use of this method for the Great Lakes. With the increased sensitivity of liquid scintillation techniques, incubation times can probably be reduced to 1.5 hr so that a number of stations could be processed in the same day.

The fact that constant values of the extinction coefficients were used for the model calculations need not necessarily be a serious drawback. For example, if future investigations indicate that the offshore extinction coefficient on a given date is 0.15, then the output from the present calculations can be corrected by multiplying the answers by $0.2 \div 0.15$. The inshore situation is not so simple since the precise values of the extinction coefficients will not likely follow any simple seasonal pattern but will instead depend upon currents and winds, which can cause rapid and massive movement of waters in this region. The stochastic nature of storms which produce wave scouring along the shore, producing suspended clay particles and the possibility of marl precipitation in regions of rapid photosynthesis, could also cause transparency to vary rapidly in the inshore areas. It must be admitted that, for this and other reasons, the model output is less credible for station 1 than for any of the others.

It is of interest to examine one more function that can be derived from the model output. Multiplying both sides of equation [3] by the extinction coefficient gives

$$F(I/I_k) = \frac{\epsilon \sum\sum P}{\lambda P_{opt}} = \frac{\delta}{2}\left[\int_{-1}^{1}\int_{0}^{I_0(x)/\delta I'_k} \frac{dy}{[(1+y^2)(1+(ay)^2)^n]^{1/2}}\,dx\right] \quad [4]$$

This function is simply a change of the ordinate in Fig. 16 and is therefore plotted there. It is the same for all stations.

Vollenweider (1958) was the first to explicitly state the general relation

$$\sum\sum P = \frac{\lambda P_{opt}}{\epsilon} F(I/I_k),$$

modifying his notation to conform to that used here. Later Vollenweider (1960) showed that the models of Steemann-Nielsen (1952), Talling (1957a), Ryther (1956), and Ryther et al. (1957) could also be expressed in this form. From empirical measurements in Lake Erken (Sweden), Rodhe et al. (1958) proposed that $2.4 \leq F(I/I_K) \leq 2.7$, and was largely independent of variations in surface irradiance. They hypothesized that a value of 2.5 could be used for predictive purposes. Their limiting values are plotted in Fig. 16 as dashed horizontal lines. The average value of $F(I/I_k)$ for Lake Michigan was 2.3. It is obvious that the temporal variability of this function is too great to propose that this can be used for predictive purposes on any given date. Moreover, Vollenweider's range of values is too small. Other empirical investigations by Saunders et al. (1962) showed that the mid-

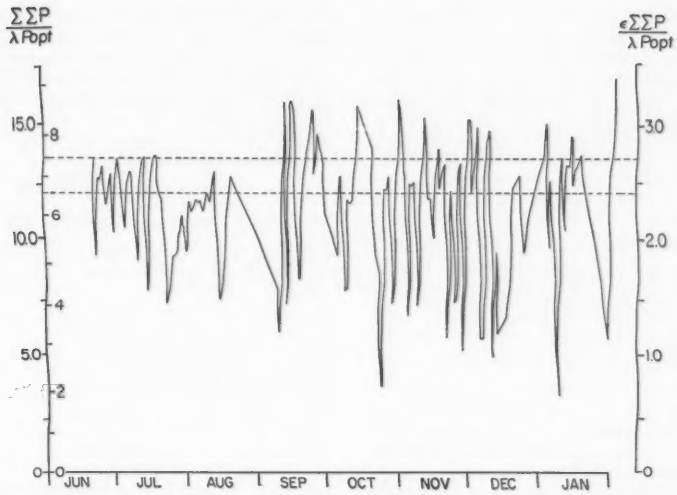

FIG. 16. Photosynthetic ratios derived from the model output, June–February. Three curves are plotted as one since the ratios differ only in scale. Left ordinate: ratio of daily integral production to λP_{opt}, the outer scale being for offshore stations and inner one for station 1. Right ordinate: ratio of the two variables described above multiplied by the extinction coefficient (called $F(I/I_k)$; it is the same for all stations). The dashed horizontal lines delimit the range $2.4 \leq F(I/I_k) \leq 2.7$, which was previously thought to include all likely values of this function.

summer mean value for Grand Traverse Bay was close to 3.3, which is about the maximum approached by my results. Vollenweider (1965) interpreted Rodhe's (1965) data and found that for a broad spectrum of lakes, the average value of $F(I/I_k)$ was close to 2.3. Vollenweider (1965) futher showed theoretically that $F(I/I_k)$ could vary up to 3.4 with reasonable assumptions for the model parameters. Again, my data support his result. The important conclusion to be drawn from Fig. 16 however, is that this function is too variable for a mean value to have any predictive power for any specific date with an accuracy of more 50%. On an annual basis such a calculation may, however, be reasonable. For any shorter time interval the full model should be used, even if the values of a, n, and I'_k must be assumed, since most of the variability of $F(I/I_k)$ is due to day-to-day variations in surface irradiance and not to the variations in the model parameters.

USES OF THE MODEL IN ENVIRONMENTAL PREDICTION AND MANAGEMENT

It is reasonable to ask what direct assistance the foregoing results can render to the solution of present environmental problems of Lake Michigan. To the extent that we need documentation of the nature and extent of the deterioration of the water quality near a large metropolitan area and some notion of the seasonal variability in reasonably undisturbed areas, this information is invaluable. It is apparent that in virtually every quantitative property the inshore Wisconsin station (near Milwaukee) was different from the other four stations. The inshore Michigan station — located far from any major population center — is very similar to the offshore stations in the rate of carbon uptake, both volumetric and integral. Moreover, by considering all of the major variables influencing carbon uptake, the model provides a single number that has valuable comparative uses. Thus, the model is a quantitative tool that can be used to objectively compare the present condition with future situations to demonstrate the effect of remedial measures or of further deterioration. It is evident that looking at any single parameter, such as P_{opt}, gives a very misleading picture of the total carbon fixation at any given point on the lake.

Since the model does not at present incorporate nutrient limitation effects directly, it is not possible to predict what effect a specific nutrient loading

rate into Lake Michigan will have on primary production. However, if the relation between P_{opt}, n, and I'_k and nutrients could be formulated through experimental means, then the model would have predictive power. In fact, it appears that this is the most fruitful path to explore in trying to incorporate nutrient effects into numerical models.

Conclusion

The model has been shown to be of value in assessing the relative importance of many factors, each of which has an influence of the magnitude of integral primary production. The decreased integral production during midsummer showed clearly that changing physiological parameters were most important at this time. The analysis indicated that the decrease was due not only to a decrease in P_{opt} but also to a decrease in the efficiency of carbon uptake at low light intensities. Another situation that the model helped to interpret was the winter condition of low solar energy input, with high efficiencies of carbon uptake at low light intensities and constant values of P_{opt}. The model showed that the decreased isolation was the most important factor since integral production decreased to the lowest observed values at this time. Thus, during summer, integral production is limited by biological factors which are probably linked to nutrients (Fee 1971a), while physical factors are of the greatest importance in the winter. Such a clear and unambiguous statement would be difficult to make solely on the basis of in situ measurements.

Perhaps the most striking fact resulting from the use of the model is that the daily variation in integral production at a single station — due simply to day-to-day changes in the pattern and quantity of surface irradiance — is nearly as great as seasonal changes. This casts grave doubts on the utility of weekly or monthly in situ experiments for elucidating seasonal trends. The model developed in this work is probably the most economical and realistic method of estimating total energy inflow through the primary trophic level.

In conclusion, the main use of the model in its present state is in the development of a sound theoretical base for studying the ecology of lakes by bringing together all the main factors influencing photosynthesis in an empirical but practical way. Ecologists can hope to realistically estimate the annual rate of energy fixation at the primary trophic level without expending excessive effort conducting daily in situ experiments. No previous model has incorporated the actual distribution of sunlight over the course of a day. Thus, the model accommodates the actual variations of the most important physical factor affecting photosynthetic carbon uptake by phytoplankton; the resulting model is quite general and Vollenweider (1970) has recommended that the method developed here be adopted as standard.

Acknowledgments

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Appendix 1

Definitions of symbols.

Symbol	*Explanation*
t	time (minutes)
λ	day length (minutes)
a, n	dimensionless parameters of the model (see text)
P	rate of photosynthesis (mg C/m^3 per min)
P_{max}	maximum rate of photosynthesis when $a = 0$ or $n = 0$ (see Fig. 1) (mg C/m^3 per min)

P_{opt}	experimentally measurable optimum rate of photosynthesis (see Fig. 1) (mg C/m^3 per min)
I	irradiance (ly/min)
I_{opt}	irradiance at which P_{opt} occurs (ly/min)
I_k	light saturation parameter when $a = 0$ or $n = 0$ (see Fig. 1) (ly/min)
I'_k	experimentally measurable light saturation parameter (see Fig. 1) (ly/min)
$I_0(x)$	surface irradiance at normalized time $x = 2t/\lambda$ (ly/min)
ϵ	extinction coefficient (meters^{-1})
δ	dimensionless auxilliary parameter defined as $P_{max} = \delta P_{opt}$ and $I_k = \delta I'_k$ (see text)
x, y	dummy variables
z	depth (meters)

Modelling Primary Production in Water Bodies: A Numerical Approach that Allows Vertical Inhomogeneities

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FEE, E. J. 1973. Modelling primary production in water bodies: a numerical approach that allows vertical inhomogeneities. J. Fish. Res. Board Can. 30: 1469–1473.

A new model for computing integral daily phytoplankton primary production is described. The model incorporates vertical variations of algal biomass, complex photosynthesis vs. light responses, nonexponential extinction of light vs. depth, and any distribution of surface light over a day. The basic approach is to combine measured relations for photosynthetic rate vs. light, light vs. depth, and light vs. time in an interpolative scheme rather than attempting to fit equations to the data and using the resulting equations to obtain a mathematical solution. The model is general and should have wide applicability. Model predictions agreed well with in situ measurements of production.

FEE, E. J. 1973. Modelling primary production in water bodies: a numerical approach that allows vertical inhomogeneities. J. Fish. Res. Board Can. 30: 1469–1473.

L'auteur décrit un nouveau modèle servant à calculer la production primaire journalière intégrale du phytoplancton. Ce modèle incorpore les variations verticales de la biomasse algale, même les réponses complexes de photosynthèse vs lumière, l'extinction non exponentielle de la lumière vs profondeur et la distribution de la lumière en surface, quelle qu'elle soit, au cours d'une journée. L'approche de base consiste à mesurer et à combiner les relations taux de photosynthèse vs lumière, lumière vs profondeur et lumière vs temps dans un schème d'interpolation, plutôt que de tenter d'appliquer les équations aux données et d'utiliser les équations qui en résultent pour obtenir une solution mathématique. Ce modèle a un caractère général et devrait avoir une application étendue. Les prédictions obtenues à l'aide du modèle s'accordent bien avec des mesures de production in situ.

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IN an earlier paper (Fee 1973) I proposed a model for obtaining estimates of integral daily phytoplankton production based on the use of a light–incubator to measure the photosynthesis vs. light response of an algal community. By also measuring the extinction of light over depth and the distribution of light over time, mathematical techniques were used to estimate production.

The model had one critical flaw: it could be used only for vertically homogeneous water masses, where the photosynthesis vs. light function and the vertical extinction of light were uniform at all depths. This paper extends the model to deal with vertical variations of the photosynthesis vs. light response and an arbitrary vertical light extinction curve. Further, the new model treats the photosynthesis vs. light function in a much more realistic manner. Predictions using the new model are compared with in situ measurements in lakes in the Experimental Lakes Area of the Freshwater Institute, northwestern Ontario.

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REQUIREMENTS FOR USE OF THE NEW MODEL

The procedure assumes only that the photosynthesis vs. light function at any depth is invariant over the time period being integrated, which is normally the lighted part of a day. Measurements of three empirical functions are needed to apply the model: (1) P(I), the photosynthesis vs. light function; (2) $\varepsilon(z)$, the extinction of photosynthetically available light as a function of depth; and (3) $I_0(t)$, the distribution of surface light over the time interval to be integrated. A digital computer is needed to perform the calculations.

CALCULATION PROCEDURE

The model simply combines the three empirical functions in a straightforward interpolation scheme. Figure 1 shows how these curves are used to simulate the in situ production vs. depth curves for two values of surface irradiance in a stratified waterbody. The first step is to divide the water column into a large number of vertical intervals (forty were

used in the test of the model). The percent of surface light that penetrates to each of these depths is calculated by exponential interpolation in the $\varepsilon(z)$ function. The P(I) function applicable to each depth is also determined at this stage. In the general case the biomass and species composition are vertically stratified and P(I) functions must be determined for enough depths to define these variations. In Fig. 1 the lake is divided into two layers, epilimnion and hypolimnion, but the model is general and will allow any number of layers.

For each value of $I_0(t)$, the intensity of light at each of the depths is calculated by multiplying the surface value by the percent that reaches each depth (ε (z)). Corresponding to the light at each depth and time, the rate of photosynthesis is calculated through linear interpolation in the P(I) function applicable to that depth. All of the rates in the water column for a given value of $I_0(t)$ are numerically integrated to give the instantaneous rate per unit area. Numerical integration of these depth integrals gives the daily rate per unit area.

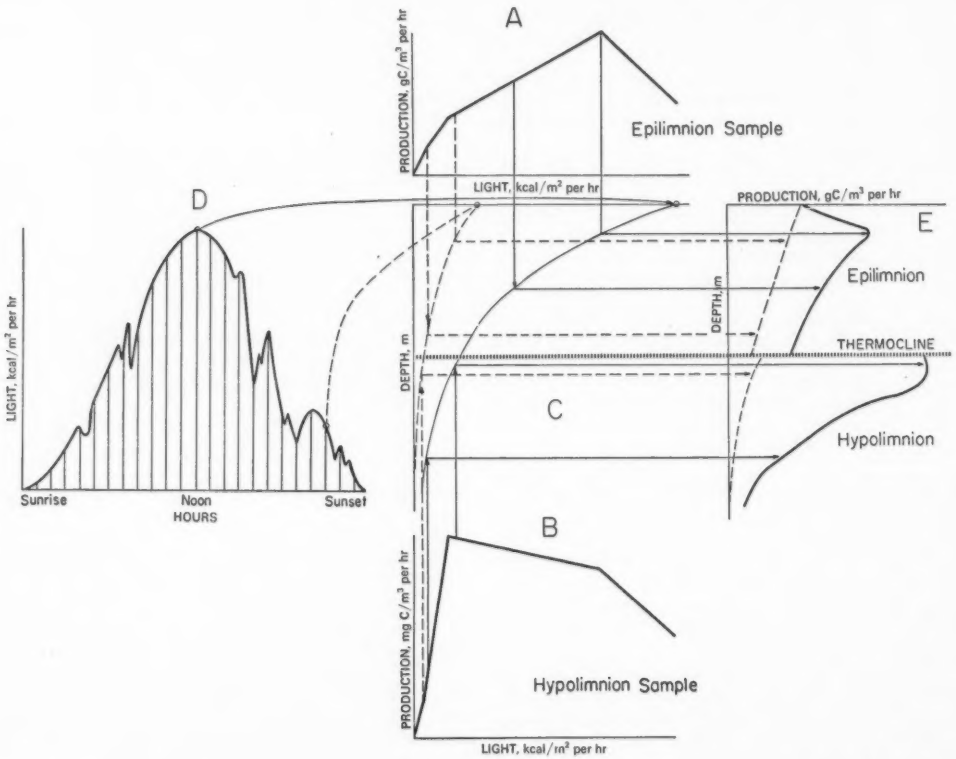

FIG. 1. The method of synthesizing the three empirical functions — photosynthesis vs. light (panels A and B), extinction of light vs. depth (panel C), and surface light vs. time (panel D) — numerically to simulate in situ photosynthesis vs. depth profiles (panel E) in a two layered system. The thermocline, which separates the two layers, is denoted by heavy dashed lines in panels C and E. For each digitized value of surface irradiance (represented by vertical lines in panel D), the following steps are performed: (1) the distribution of irradiance over depth is computed (panel C); (2) at each depth the photosynthetic rate is calculated by linear interpolation in the incubator curve (panels A and B) applicable to that depth and plotted on panel E; the lines from the incubator curves (A and B) to the light extinction curve (C) and then to the photosynthesis vs. depth curve (E) illustrate this process for a few representative depths at a high (solid lines) and a low (dashed lines) surface irradiance; (3) integration of the resultant curve gives the instantaneous rate of photosynthesis in the water column. Integration of these instantaneous rates for all values of surface irradiance gives the daily rate. The lake can obviously be divided into more layers if necessary.

A Fortran computer program which performs these calculations is presented by Fee (1973a).

One problem remains when using empirical data rather than a fitted equation to describe photosynthesis as a function of light. Usually the highest irradiance obtained in an incubator is considerably lower than full sunlight. Thus, the question of how to estimate production at irradiances above the highest incubator irradiance must be resolved. Strickland (1960), in a review of phytoplankton production, found that most algae show similar responses at very high irradiances. Optimum rates of production usually occur at 0.10–0.15 ly/min^{-1} (1 ly = 1 cal. cm^{-2}) (in the visible portion of the spectrum only). Above the optimum irradiance, photosynthesis decreases until nearly all production is stopped at and above an irradiance of 0.50 ly/min. Figure 2 shows how the model uses this information. The equation used is

$$P = P_H \left[1 - \frac{I - I_H}{.5 - I_H} \right]^q, \quad I_H \leq I \leq .5$$

$$P = 0 \quad , I > .5$$

where I is the irradiance (ly/min), P is the computed production at the irradiance I, P_H is the observed production rate in the highest irradiance compartment of the incubator, I_H is the irradiance of the highest irradiance compartment of the incubator, and q is an exponent which may be varied to give different shapes to the curve. In the modelling done for this work, q was arbitrarily set to two. Other choices for this exponent do not alter the results by more than a few percent becauce these high irradiances occur at only very shallow depths and do not influence the rates of production at greater depths, where most of the production occurs.

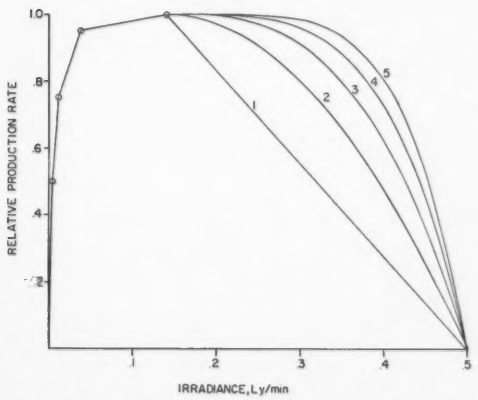

FIG. 2. This figure shows the method used to determine photosynthesis rates for irradiances higher than the highest irradiance level obtained in an incubator. The circled points represent a typical set of experimental results; the curves at irradiances higher than 0.15 ly/min are generated with the equation given in the text. The numbers to the right of the curves correspond to different values of the exponent.

Methods of Verification

The model was tested by simulating production in ELA lakes in which in situ experiments were being performed. In situ production was measured by the method of Schindler and Holmgren (1971); carbon uptake was measured with the acidification and bubbling method (Schindler et al. 1972) instead of filtration.

Light extinction in the lakes (the $\epsilon(z)$ function) was measured with a Whitney model LMT-8A submersible photometer (cadmium sulfide cell). Solar radiation (the $I_0(t)$ function) was monitored with an Eppley Precision Spectral Pyranometer at the ELA meteorological station. The strip chart output from the solarimeter was digitized at 10-min intervals with a d-Mac mechanical digitizer together with the computer program presented by Fee (1971a).

Phytoplankton samples for incubator experiments to determine the P(I) function were taken with a Van Dorn sampler and were transported to the laboratory in ½-gal opaque polypropylene bottles. The 125-ml Pyrex experimental bottles were filled from these with a siphon. Care was taken to exclude bubbles from the sample bottles. Duplicate 50-ml polypropylene syringe samples for dissolved inorganic carbon (DIC) analysis were drawn from the sample bottles immediately prior to filling the experimental bottles. The DIC levels were measured with a gas-chromatographic technique (Stainton 1973). From 1 to 5 μC of radioactive bicarbonate tracer were added to the experimental bottles to obtain desired sample activities. An incubator similar to that described by Fee (1973) was used to determine carbon uptake as a function of light. Duplicate experimental bottles were placed in each of four chambers having irradiances of 0.14, 0.037, 0.011, and 0.0038 ly/min (in the visible portion of the spectrum), respectively. Light was supplied by a bank of six 500-w tungsten in iodine vapor bulbs. The spectral distribution of light energy was very similar to that presented by Fee (1973a). The bottles were attached to plexiglass discs in the four experimental chambers, vertically rotating at a velocity of 5 rpm. The quality and quantity of light in the chambers were monitored with an ISCO spectroradiometer. Incubation times varied from 2 to 4 hr near midday.

Results and Discussion

The agreement between observed and predicted production rates in all lakes is very good (Fig. 3) and the model is shown to be a suitable substitute for in situ measurements.

The new model is easier to apply than other mathematical models because it does not require any preliminary manipulations of the data. The most difficult part of the models of Vollenweider (1965) and Fee (1971) was fitting a nonlinear equation with four parameters to incubator data relating photosynthesis to light. Vollenweider did not attempt the solution; Fee derived valid statistical techniques for this purpose but the method required an elaborate computer program and is too complex for most purposes. Moreover, data of the sort shown in Fig. 4, which result from the presence of algae with different light adaptations in the same sample, cannot be accurately fitted with any of the previously available photosynthesis vs. light equations. Such data present no problem with the new model. Finally, the cost of the computations is much less with the new model since the old models require the repeated evaluation of mathematical functions such as exponentials and square roots while the new model uses the much faster process of linear interpolation.

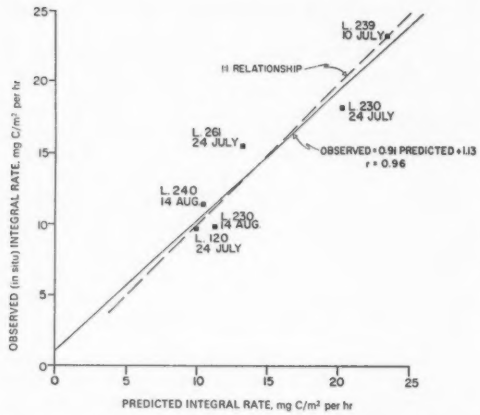

FIG. 3. The relation between model predictions and observed rates of integral production in lakes in the Experimental Lakes Area during the summer of 1972.

The new model is more accurate and flexible than earlier approaches since it can be used even when vertical distribution of algal biomass is not uniform and the extinction of photosynthetically available light is nonexponential.

In comparison to the in situ method, the main advantage obtained by using the new model is the greater accuracy with which seasonal and annual rates of primary production may be estimated, since production for all variations of surface irradiance can be accurately calculated without making continuous in situ measurements. Futher, as demonstrated by Fee (1973) and Schindler and Fee (1973), the incubator data are much more readily interpreted in a causal manner than in situ data. Acclimation of the photosynthetic community in response to seasonal changes of surface irradiance, water temperature, and nutrient availability can

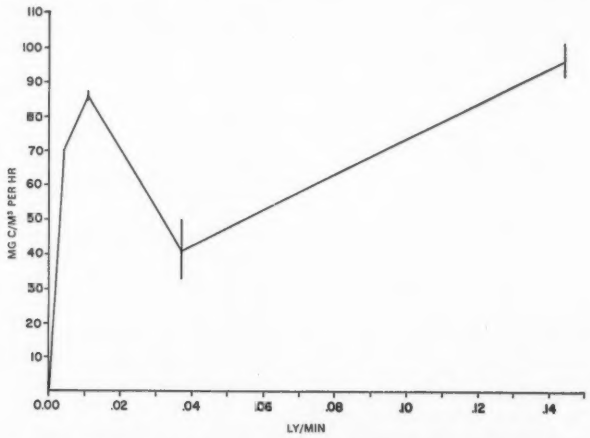

FIG. 4. Atypical experimental results obtained with the phytoplankton incubator from lake 304 (epilimnion sample, September 21, 1972). Vertical lines on the graph connect duplicate results at each irradiance. Data of this kind are not easily modelled mathematically but are simple to use in the numerical approach.

be studied much more easily when samples are incubated under standard light conditions. It is also easier to quantitatively compare the photosynthetic limitations of algal communities in different water bodies or different parts of a single water body with such data.

Decisions that must be made before applying the technique are the number of samples needed to define vertical variations of biomass and the number of light intensities needed in the incubator system to define the production vs. light function. Ideally one would use a continuous flow fluorometer to map the in situ distribution of chlorophyll in the water column (Berman 1972) and take samples accordingly. Lacking the rather elaborate apparatus required for this procedure, it is probably safe to assume that the epilimnion, metalimnion, and upper hypolimnion are well-defined layers each containing homogeneous phytoplankton populations, and three samples would be adequate to define the major biomass changes in the water column. The number of light intensities in the incubator is limited by the practical problem of sample processing; four light levels were used for the modelling done to verify the theory presented in this paper.

As previously mentioned, the only theoretical assumption made for the model is that there is no variation of the photosynthesis vs. light function at a given depth over the time period covered by the integration. Changes over the daylight period due to the depletion of some substance required for growth can introduce serious errors. Schindler and Fee (1973) studied a highly eutrophic softwater lake in which a deficit of inorganic carbon severely affected the production vs. light function over the course of a day. However, in such systems the diurnal changes of CO_2 are directly usable for estimating primary production and there is no need to model the system. In the vast majority of lakes this is not a problem and the model should be a useful tool for extending productivity surveys and for generating realistic input to more inclusive limnological models.

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Gas-Exchange Rates in a Small Lake as Determined by the Radon Method[1]

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EMERSON, S., W. BROECKER, AND D. W. SCHINDLER. 1973. Gas-exchange rates in a small lake as determined by the radon method. J. Fish. Res. Board Can. 30: 1475–1484.

The radon method, used previously in ocean-atmosphere systems, is used here to determine the gas-exchange rate between the atmosphere and lake 227 of the Experimental Lakes Area. Fertilization of the lake with nitrogen and phosphorus caused the carbon dioxide partial pressure in the lake water to drop well below atmospheric levels; hence, in order to better understand the carbon budget of the lake, an estimate of the CO_2 gas-exchange rate was necessary.

To determine gas-exchange rates by measuring radon evasion to the atmosphere the source of radon in the lake water must be dissolved radium. Since the radon concentration in lakes derives not only from the decay of dissolved radium but also from the inflow of radon-rich groundwaters, radium was added to the lake to increase the radon concentration well above this fluctuating background level. Although this procedure was complicated by algal uptake of the radium in the lake (Emerson and Hesslein 1973), we were able to place limits on the gas-exchange rate.

Our results indicate that the "stagnant boundary layer" thickness is approximately 300 μ. This value is among the largest observed in natural waters. Using this value and the partial pressure of CO_2 in the lake water we have calculated an invasion rate of 17 ± 8 mmoles CO_2/m^2 day.

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La méthode de détermination des taux d'échanges gazeux par le radon, appliquée antérieurement aux systèmes océan-atmosphère, a été utilisée pour mesurer les taux d'échanges gazeux entre l'atmosphère et le lac 227 de la région des lacs expérimentaux. La fertilisation du lac à l'azote et au phosphore a causé une diminution de la pression partielle du CO_2 de l'eau du lac bien au-dessous des niveaux atmosphériques; il a donc fallu obtenir un estimé du taux d'échange gazeux du CO_2 afin de mieux comprendre le bilan du C dans ce lac.

Afin de déterminer les taux d'échanges gazeux en mesurant l'échappement du radon dans l'atmosphère, la source de radon dans le lac doit être du radium dissous. Puisque le radon des lacs provient non seulement de la dégradation du radium dissous, mais aussi des eaux souterraines, riches en radon, qui entrent dans le lac, il a fallu ajouter du radium au lac, afin d'élever la teneur en radon bien au-dessus du niveau d'arrière-plan variable. En dépit de complications causées par la captation du radium dans le lac par les algues (Emerson et Hesslein 1973), il a été possible d'établir des limites pour les échanges gazeux.

Nos résultats démontrent que l'épaisseur de la "couche de démarcation stagnante" est d'environ 300 μ. Cette valeur est une des plus hautes jamais observées dans des eaux naturelles. Utilisant ce chiffre et la pression partielle de CO_2 dans l'eau du lac, nous sommes arrivés à un taux d'invasion de 17 ± 8 mmoles CO_2/m^2 par jour.

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It is well known that the atmosphere can be an important source or sink for the biologically important gases CO_2 and O_2. Its role is especially important if the chemistry of the water has been altered by eutrophication. For example, Schindler et al. (1971) and Schindler et al. (1972) have suggested that invasion of atmospheric carbon dioxide is sufficient to allow a lake to eutrophy, provided supplies of other critical nutrients are adequate. It is therefore important, to understand the factors controlling the biochemistry of a lake, to be able to measure the gas-exchange rate.

Gas-exchange rates have been estimated quantitatively using either ^{14}C or the relation between radon-222 and its precursor radium-226. These two methods have been used extensively in the ocean-atmosphere system. Craig (1957) and Munnich and Roether (1967) are two of many who have used ^{14}C. The radon method has been used by Broecker (1965), Broecker et al. (1967), and Broecker and Peng (1971). The only published studies, however, in which these isotopes were used to measure the gas-exchange rate in a lake-atmosphere system are those of Broecker and Walton (1959) and Thurber and Broecker (1970) who used natural and bomb-produced ^{14}C to determine the carbon dioxide exchange rate in the Great Basin lakes of the United States. The objective of this paper is to describe the use of the isotope pair radon-222 and radium-226 to determine the gas-exchange rate in the lake-atmosphere system.

Description of Lake

Lake 227 is a small lake (area = 5.0 ha, vol = 2.2×10^5 m^3, max depth = 10 m) located on the Precambrian Shield in the Experimental Lakes Area (ELA) of the Fisheries Research Board of Canada. Morphometry of the lake is discussed by Brunskill and Schindler (1971) and other limnological information may be found in Schindler et al. (1971 and 1973).

The lake has been fertilized with phosphorus and nitrogen as part of an experiment in eutrophication. Photosynthetic CO_2 uptake by the increased standing crop of phytoplankton caused the *p*H of the epilimnion to increase from natural midsummer levels of 6.5–7.5 to 9.5–10.2 in midsummer of 1970. Because there is no calcareous material in the bedrock surrounding the lake, and because bioassay experiments indicated that CO_2 in the lake was limiting to phytoplankton photosynthesis, the rate at which CO_2 enters at the air–water interface appeared to be a primary factor governing the carbon budget of the lake.

All components of the carbon cycle, including rates of inflow, outflow, sedimentation, and increases in seston and soluble carbon fractions are monitored regularly (Schindler et al. 1971, 1973, and unpublished data). These data made it possible to calculate the influx of CO_2 from the atmosphere by mass balance, i.e. if there is no increase in the carbon content of the water, the amount of carbon leaving the lake by sedimentation and outflow must equal the amount entering the lake from inflow and the atmosphere. Hence, lake 227 was an ideal place for determining the reliability of gas-exchange rates measured by the radon method.

We will describe here the methods used to determine the O_2 and CO_2 gas-exchange rate and the results derived. The relation between the CO_2 gas-exchange rate and the biogenic processes of the lake have been published elsewhere (Schindler et al. 1972).

Theory

In natural systems gas transfer between the atmosphere and water is most widely characterized by the use of a "stagnant boundary layer" model (Bolin 1960; Hoover and Berkshire 1969; Thurber and Broecker 1970; Quinn and Otto 1971). In this model gas exchange is pictured as diffusion of a gas through a stagnant boundary layer of variable thickness which occupies the surface of the water next to the atmosphere. Although it is unlikely that completely stagnant layers exist in nature, the model incorporates the essential features of gas–liquid transfer, that of dissolution and molecular diffusion of the gas before convective mixing in the atmosphere or in the water can become important. In this model the surface of the water (the top of the "stagnant boundary layer") has the atmospheric equilibrium concentration of the gas in question, i.e. the partial pressure (p) of the gas in the atmosphere times the solubility (α) of the gas in water. The lower side of the boundary layer has the concentration of gas in the water below it (C_O). The air above the layer and the water below it are assumed to be well mixed and gases pass through the layer by molecular diffusion only. Mathematically this model is represented by Fick's Law for diffusive flux:

$$F_D = -\frac{dC}{dx} D \qquad (1)$$

where F_D is the gas flux, C is the concentration of the gas at depth x in the stagnant boundary layer, and D is its molecular diffusion rate in water. Represented in terms of the stagnant boundary layer model this law becomes:

$$F_D = \frac{C_O - p\alpha}{z} D \qquad (2)$$

where z is the thickness of the boundary layer.

It is clear that in this model the hydrodynamic properties of the water as they apply to gas exchange (i.e.

agitation, surface area, etc.) are all characterized by one parameter, the stagnant boundary layer thickness (z). Because C_O, p, α, and D can be measured for most gases, it is the parameter z that we wish to find in our gas-exchange studies. Once this variable has been characterized for a water body, the diffusive flux of any gas may be calculated.

Broecker, in his 1965 paper, explains in detail how the stagnant boundary layer thickness is determined by the radon method; briefly, it is as follows. Radon-222 is a radioactive gas with a half-life of 3.85 days whose precursor radium-226 (half-life = 1600 years) exists in solution in natural waters. The partial pressure of natural radon from radium decay in most waters greatly exceeds the radon partial pressure in the overlying air. For this reason radon in the surface and near surface waters escapes to the atmosphere, leaving the surface waters depleted in radon with respect to the amount expected for secular equilibrium with the dissolved radium. By measuring and integrating the radon and radium activities as a function of depth the depletion of radon resulting from flux to the atmosphere can be estimated, allowing net gas escape rate for radon to be calculated.

The integrated radon deficiency must be related to the flux of radon through the stagnant boundary layer as follows:

$$\int_0^\infty (C^*-C_h)\lambda dh = \frac{D(C_O - p\alpha)}{z} \qquad (3)$$

(radon activity deficiency) (flux of radon through the boundary layer)

where C^* is the equilibrium activity of radon which would be supported by radium present in the water, C_h is the actual activity of radon gas at depth h below the stagnant boundary layer, and λ is the decay constant of radon.

The integral value may be reexpressed in terms of $\hbar$, the depth to which the surface radon activity would have to be extended to yield a homogeneous layer with deficiency equal to that yielded by the integral; i.e.

$$(C^* - C_O)\,\hbar\lambda = \int_O^\infty (C^* - C_h)\,\lambda dh \qquad (4)$$

and therefore

$$(C^* - C_O)\,\hbar\lambda = \frac{D(C_O - p\alpha)}{z} \qquad (5)$$

and if $C_O >> p\alpha$ (as is the case for radon),

$$\frac{D}{z} = \left(\frac{C^*}{C_O} - 1\right)\hbar\lambda \qquad (6)$$

D/z is a "mass transfer coefficient" (units of cm/sec) and may be thought of as the velocity at which a hypothetical piston rises through the water sweeping the contained gases before it into the atmosphere. The stagnant boundary layer thickness (z) can then be calculated by inserting the molecular diffusion constant for radon in water (1.36×10^{-5} cm^2/sec at 25 C, extrapolated from data at 18 C, Rona 1917).

The above calculations are sufficient for determining the gas-exchange rate for any gas which does not react with water (equation 2). The case for carbon dioxide, however, is considerably more complicated because CO_2 does react with H_2O molecules and with OH^- ions in the stagnant boundary layer forming HCO_3^- ions.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \quad \text{(dominant at } p\text{H less than 8)} \qquad (7)$$

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \quad \text{(dominant at } p\text{H greater than 8)} \qquad (8)$$

These reactions create a bicarbonate gradient across the stagnant boundary layer. The bicarbonate gradient acts as a driving force for its self diffusion in the same manner that the CO_2(aq) gradient is a driving force for CO_2 molecules. The net result is two modes of transfer of the carbon atom across the stagnant boundary layer, thereby increasing the amount of CO_2 transferred with respect to that expected for CO_2(aq) diffusion only. It is convenient to express the effect of chemical reactions in terms of an "enhancement factor" which is the ratio of the net gas flux (F_N) between the atmosphere and the water to the net gas flux were there no reaction (F_D).

$$E = \frac{F_N}{F_D} \qquad (9)$$

The enhancement factor (E) has been determined theoretically by Bolin (1960), Hoover and Berkshire (1969), and Quinn and Otto (1971). Quinn and Otto criticized the previous authors by showing that their calculations were applicable only when no net transfer of CO_2 occurs because they neglected to consider conservation of charge in the stagnant boundary layer. To simplify the calculations of their model Quinn and Otto assume that the H^+ and OH^- ions are of negligible importance for considering charge balance, which is indeed the case in sea water for which the calculation was made. Unfortunately, this assumption cannot be applied here because OH^- ion is over one half of the alkalinity in lake 227 ($OH^- = 1 \times 10^{-4}$ moles/liter and alkalinity $= HCO_3^- + 2CO_3^= + OH^- \simeq 1.75 \times 10^{-4}$ m.eq./liter). Calculation of the enhancement factor for waters in which the H^+ or OH^- ions are important for charge balance within the stagnant boundary layer (i.e. dilute lakes with a pH not near 7) involve complicated differential equations which have not yet been solved.

Because the theory describing the enhancement of CO_2 gas transfer in waters with the chemical properties of lake 227 is presently inadequate, we constructed an abiotic simulation of the lake and determined the enhancement factor empirically. The details of the experiment are presented later in this paper.

Methods

The natural radon-222 activity of the lake, as measured in July 1970, was less than 1 dpm/liter at all levels (Fig. 1). The activity of natural radon depends not only on the radium content of the water and the rate of gas-exchange but also on the rate at which radon is supplied by groundwater. Determination of gas-exchange rates using this natural tracer is not possible because the influence of the latter parameter is difficult to estimate. Instead the lake was artificially enriched with enough radium-226 to increase the radon activity to a level which would make the influence of radon from groundwaters negligible. This was accomplished by adding 9.7 mc of radium-226, bringing the theoretical activity of the epilimnion waters to a level of about 200 dpm/liter.

The radium was dispersed throughout the epilimnion of the lake by diluting a concentrated solution of radium bromide in a 50-liter container of lake water which was towed around the lake while it slowly drained. The epilimnion of the lake was then allowed to mix for 6 days before sampling. The following is an explanation of the sampling methods.

Two-liter glass bottles were evacuated in the laboratory and taken to the field. Samples were taken at depths of 0, 0.5, 1, 2, 2.5, 3, 5, 7, and 9 m by dropping a length of pressure tubing to the desired level, purging the tubing, and then attaching it to the evacuated bottle and allowing the bottle to fill with water. This method assured that any radon which escaped from the water upon filling was retained within the sampling bottle. The samples were then returned to the laboratory where helium was recirculated through the water and through liquid nitrogen-cooled traps at a rate of twelve liters per minute for 30 min. Calibrations have shown that over 90% of the radon present was recovered by this procedure (Broecker 1965).

Radium activities were determined by the same extraction procedure after the sample bottles were sealed and stored for a minimum of 4 days while the radon grew toward secular equilibrium with its parent radium. It should be noted here that the radium activities of the lake between August 8 and August 25, 1970 must be considered minimum values. The water samples for these profiles were stored in polyethylene containers for several weeks and then transferred to glass sample bottles for radium analysis, a normal procedure when determining the radium activity of sea water. This procedure proved inadequate for lake 227 water which is very low in electrolytes (the conductiity is about 20-μmho/cm, Armstrong and Schindler 1971) and has a high content of dissolved organic matter. The lack of carrier ions in the water in addition to sedimentation of the organic material caused a significant amount of the radium ion to adsorb on the sides of the polyethylene containers. Analyses on empty containers showed that up to 40% of the total radium remained on the sides in some cases. Water poured from them thus had lower than true radium activities. When this problem was discovered subsequent samples for radium were kept in their original glass bottles and acidified immediately after radon analysis.

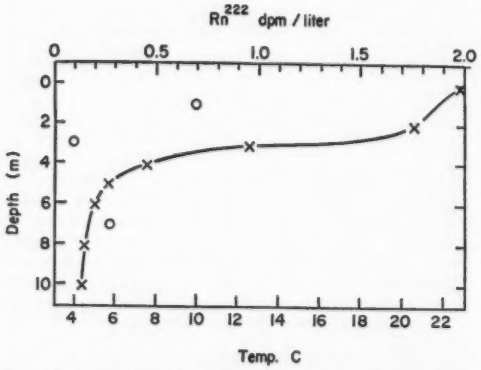


FIG. 1. Natural radon and temperature concentrations in lake 227 on July 21, 1970 (6 days prior to spiking). 0 = Rn; x = temp.

Using the radon gas exchange data derived from the above measurements the O_2 exchange rate and the diffusive portion (F_D) of the CO_2 invasion rate can be calculated. To determine the chemical enhancement factor for carbon dioxide exchange the simulation experiment was employed. The objective was to recreate, in an abiotic environment, the chemical and physical parameters of lake 227 which are important for gas exchange. The CO_2 invasion could then be determined by the radon method and by measuring the increase in the carbon concentration of the water with time. The difference between these two values is the chemical enhancement. The following procedures were used in this experiment.

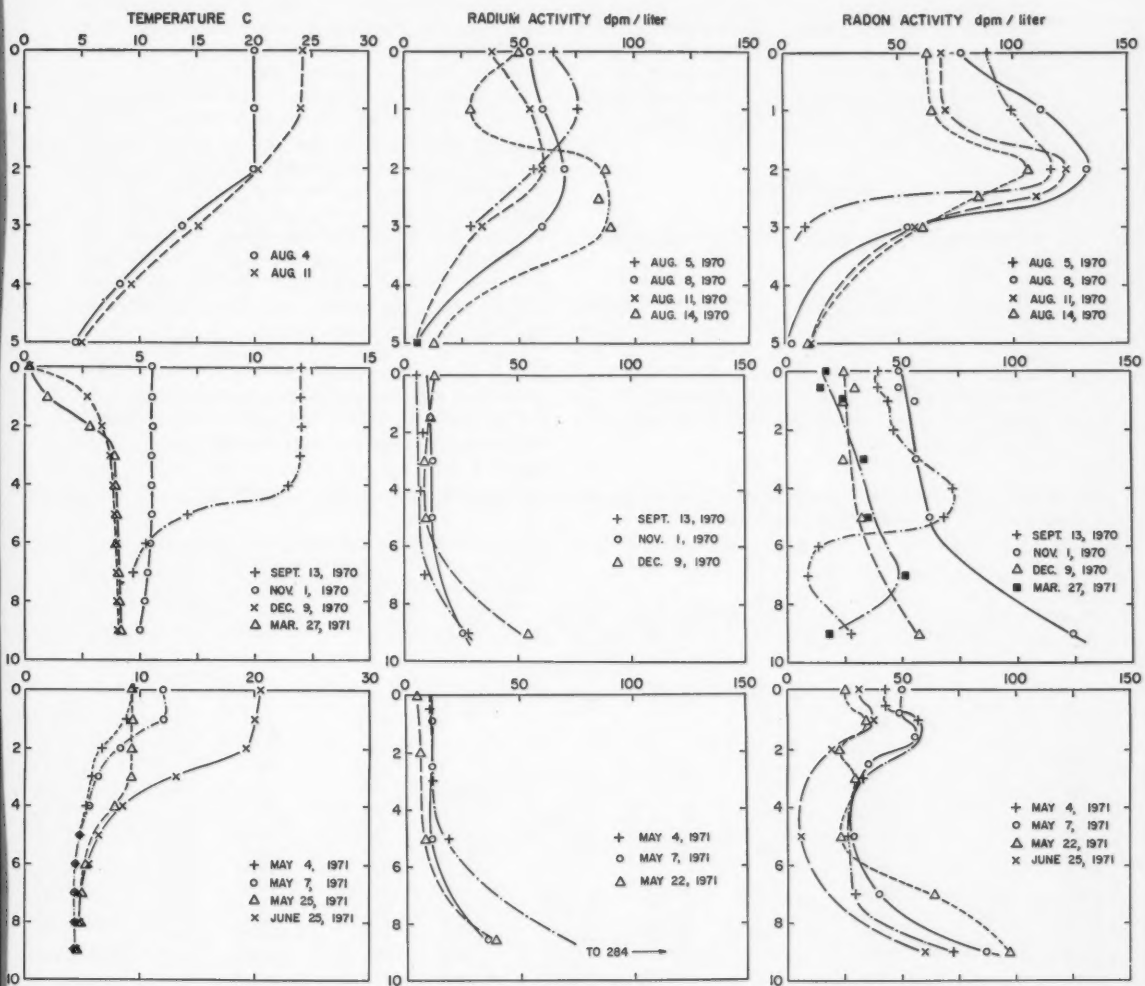
A polyethylene tank 2 m in diameter was filled to a depth of between 8 and 10 cm with distilled water and sheltered from direct sunlight. To establish the physical conditions for gas exchange analogous to those of lake 227, the stagnant boundary layer thickness which was determined for the lake was as nearly as possible recreated in the tank water. This was accomplished by adding radium and adjusting the radium/radon ratio by controlling the amount of agitation of the water. When the physical state of the water was appropriately established, the alkalinity of the lake (150–200 $\times$ 10^{-6} meq/liter) was reproduced in the tank water by adding KOH. Following the addition, the pH rose to approximately 10, lowering the p_{CO_2} of the water well below the atmospheric equilibrium concentration. The invasion of CO_2 was then monitored by measuring the increase in the dissolved inorganic carbon content ($\Sigma CO_2 = HCO_3^- + CO_3^= + CO_2(aq)$) of the water using a gas chromatograph. Temperature, Rn, Ra, and water depth (h) were also monitored as CO_2 invaded from the atmosphere.

Results

Radon, radium, and temperature profiles for the sampling dates of August 1970 through June 1971 are shown in Fig. 2. It is apparent from these data that the radium activity 1 month after the addition

is greatly depleted from the expected value of 200 dpm/liter and that there is a large amount of unsupported radon in the lake (unsupported radon = radon in solution — radium in solution). These observations, in addition to the lack of any large buildup of radon and radium below the thermocline until after fall turnover, indicate that the radium was nearly all taken up in the littoral zone of the lake. (The inaccuracy of the August radium profiles obscures any precise knowledge of the radium concentration before September 13.) Further investigation into the location and migration of the radium in the lake is presented elsewhere (Emerson and Hesslein 1973).

Because the radium was taken up the radon profiles are not a simple combination of in solution radon production, decay, and flux to the atmosphere. The radon is produced in the littoral regions and diffuses toward the interior of the lake while it decays and escapes to the atmosphere. Although



IG. 2. Profiles of radon, radium, and temperature for the period August 5–14, 1970 (*top*), September 13, 1970–March 27, 1971 (*center*), nd May 4–June 25, 1971 (*bottom*).

these complexities preclude a precise determination of the diffusive gas-exchange rate we have been able to place limits on the value.

To evaluate the chemical enhancement factor the simulation experiment was carried out. When the *p*H and alkalinity of the water were fixed by adding OH^- ion, the increase in DIC was measured with time. The *p*H and CO_2(aq) were then calculated for each DIC measurement. The results of the experiment are shown in Table 1. It should be mentioned that the DIC concentration is above the atmospheric equilibrium value after 2236 min. We believe that this is the result of microorganisms which had grown in the water. Fortunately, as we shall see later, the only important part of the experiment for our purpose is the first 1000 min during which the water was most probably abiotic.

Conclusions

Determination of Mass Transfer Coefficient (D/z)

Because the radium did not remain in solution the value of the equilibrium epilimnion radon activity (C*) is uncertain. To place limits on this value, and consequently on the radon flux to the atmosphere, we have used the knowledge of the absolute amount of radium added to the lake and the radon profiles.

We estimate a maximum flux to the atmosphere by integrating the radon profiles of August 8, 11, and 14 to obtain the total amount of radon in the lake water at these three samplings. The average value of these three profiles is then subtracted from the total amount of radon which would be produced by the radium which was added (see Emerson and Hesslein 1973). We then assume that (1) all the missing radon ($\sim 10 \times 10^9$ dpm) has escaped to the atmosphere and, (2) that if the radium had remained in solution its activity, like temperature (see Fig. 2), would be constant to about $1\frac{1}{2}$ m. If this is the case, then the equilibrium radon activity (C*) i.e. that if there were no gas exchange, must be roughly given by the curve labelled case 1 in Fig. 3.

The minimum value (case 2 in Fig. 3) is estimated by taking the average of the three August profiles and assuming that the equilibrium radon activity (C*) of the epilimnion is equal to the highest observed radon activity (i.e. that at the base of the epilimnion). This is done because the smallest amount of gas exchange to the atmosphere will have occurred from waters just above the thermocline. This estimate of C* must be a minimum because it may be invalid to assume that there has been no exchange from this level.

The data of August 8, 11, and 14, 1970 are used for the above determinations because these profiles were taken after the radon had reached equilibrium and became well mixed, and before vertical mixing spread the radium to greater depths, factors which

Table 1. Results of simulation experiments. *z* was calculated from the Ra/Rn ratio and h; CO_2(aq) and *p*H were calculated from Alk and ΣCO_2. (See text for explanation.)

Time (*min*)	h (*cm*)	Temp. (*C*)	Rn (*dpm/liter*	Ra (*dpm/liter*)	Ra/Rn	*z* (*cm*)	Alk (10^{-6} *meq/liter*)	ΣCO_2 (10^{-6} *moles/liter*)	CO_2(aq) (10^{-6} *moles/liter*)	*p*H
				Experiment – 1						
0	10.2	23.3	99	2150	21.7	.0235	184	50	00.0	10.07
62		23.0					184	59	00.0	10.03
125		23.5					184	63	00.0	10.01
309		24.4					184	103	00.0	9.78
648		23.8	122		18	.0290	184	146	00.1	9.39
880		22.1					184	148	00.1	9.36
1138		20.5					184	165	00.3	9.06
1371		18.5	109	2250	20.6	.0225	184	179	01.2	8.55
1530		18.0					184	179	01.2	8.55
1702		17.9					184	187	04.6	7.97
1881	10	17.9					184	189	06.2	7.84
2236		15.6	80	2200	28.5	.0185	184	204	20.4	7.33
2732		13.0					184	210	26.3	7.22
3081		15.7	140	2170	15.5	.0340	184	208		
3448		18.0					184	207	23.3	7.27
4400	9.5	17.5					184	217	33.2	7.22

cause further uncertainties in the calculations. The curve labeled "Obs." (Fig. 3) is the average of these three profiles.

Estimates of radon loss to the atmosphere for cases 1 and 2 are presented in Table 2. The mass transfer coefficients corresponding to cases 1 and 2 are presented in the following table:

Parameters used in piston velocity calculations (average of August 8, 11, 14, 1970 profiles).

Case	$C^\circ\lambda$ dpm/liter	$C^*\lambda$ dpm/liter	$\frac{C^*}{C^\circ} - 1$	h m	λ year^{-1}	D/z m/day	D/z m/year
1	70	216	2.04	1.75	63	.62	215
2	70	120	0.7	1.3	63	.15	57

If these assumptions are valid, the mass transfer coefficient determined for August 1970 is between 0.15 and 0.6 m/day (55 and 220 m/year). The corresponding limits on the stagnant boundary layer thicknesses (z) are 780 and 195 μ. We take 0.40 ± 0.20 m/day and hence a value of 300 μ (with an estimated uncertainty of +300 μ, −100 μ) as our best estimate of the actual mass transfer coefficient and boundary layer thickness for the August 1970 period.

CO_2 and O_2 Partial Pressures

To calculate the gas-exchange rates for CO_2 and O_2 using the stagnant boundary layer thickness (z), the partial pressure difference of these gases between the atmosphere and the lake is essential. CO_2 and O_2 partial pressure data, measured weekly at 1000 hr, in lake 227 are available for the summer of 1970. Recent hourly measurements (Schindler 1972 unpublished data) of these two gases, however, show that there is a large diurnal fluctuation of O_2 and CO_2, indicating that once a day measurements are not representative of the whole day.

The diurnal fluctuation is caused by photosynthesis and respiration of the large standing crop of plankton in the lake. The CO_2 concentration varies from 10 to 50 μmoles ΣCO_2/liter and O_2 varies between 300 and 340 μmoles O_2/liter. Because the lake has continually received nutrient addition during the last 2 years one cannot assume the 1972 diurnal concentrations to be the same as those of 1970. The lake had a higher standing crop of phytoplankton in the summer of 1972 and consequently the fluctuations were probably greater then than they were in 1970.

The lack of diurnal partial pressure data for 1970 makes the direct calculation of the gas-exchange rate for O_2 impossible. Since the O_2 partial pressure was near or greater than the atmospheric equilibrium

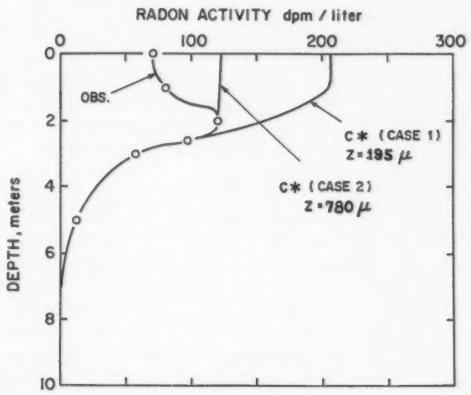


Fig. 3. Measured radon curve and limiting radium activity curves for August 8–14, 1970 (see text).

Table 2. Estimates of radon loss to atmosphere (average of August 8, 11, 14, 1970) profiles.

Depth interval meters	Volume 10^7 liters	Obs. Rn activity 10^9 dpm	Equilibrium Rn activity		Rn lost to atm	
			Case 1 10^9 dpm	Case 2 10^9 dpm	Case 1 10^9 dpm	Case 2 10^9 dpm
0–1	4.6	3.6	9.8	5.6	6.2	3.2
1–2	3.7	3.8	6.9	4.4	3.1	1.4
2–3	3.1	2.8	3.0	3.1	0.2	0.3
3–4	2.7	1.0	1.0	1.0	0.0	0.0
4–5	2.5	0.3	0.3	0.3	0.0	0.0
5–6	2.1	0.1	0.1	0.1	0.0	0.0
Σ	–	11.6	21.1	14.5	9.6	4.9

value ($\alpha_{O_2}p_{O_2} = 1.26 \times 10^{-3}$ moles O_2/liter atm, at 25 C, Handbook of Physics and Chemistry 1954, $\times$ .21 atm = .266 m moles O_2/liter) the true average value for 1970 is necessary in the gas-exchange calculations. This is not the case for carbon dioxide. The CO_2 partial pressure (CO_2(aq)) in lake 227 fluctuates between 1.5 and 2 orders of magnitude below the atmospheric equilibrium concentration ($\alpha_{CO_2}p_{CO_2} = 3.42 \times 10^{-2}$ moles CO_2/liter atm, Buch 1951, $\times$ 320 $\times 10^{-6}$ atm = 11 µmoles CO_2/liter). Since the CO_2 increase because of respiration in 1970 was probably less than in 1972, the CO_2 partial pressure in the lake was always negligible compared to the atmospheric equilibrium value. Hence the exact CO_2(aq) of the lake is not needed for the calculation.

For this reason the CO_2 gas-exchange rate will be calculated first and then an indirect attempt at evaluating the O_2 gas-exchange rate will be made.

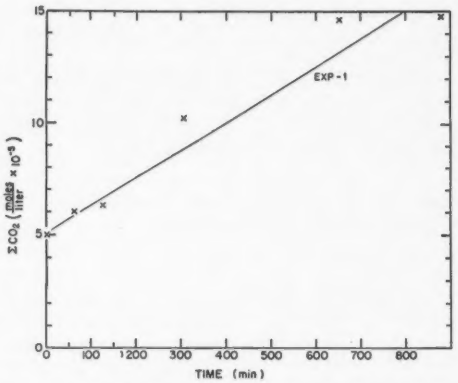


FIG. 4. DIC (ΣCO_2) increase vs. time in the simulation experiment. (The distilled water had an initial ΣCO_2 of 50 mmoles/liter. See text for explanation of the experiment.)

CO_2 Gas-Exchange Rate

The molecular diffusion constant for CO_2 is 1.91×10^{-5} cm²/sec (Cullen and Davidson 1957). Using this value, a stagnant boundary thickness of 300 µ (+300 µ, −100 µ), and the atmospheric equilibrium CO_2 concentration, an invasion of 6 ± 3 mmoles/m² day is calculated, without correction for chemical enhancement. This value is the diffusive portion (F_D) of the total gas-exchange rate. For the purpose of determining the chemical enhancement of lake 227, the only relevant part of the simulation experiment (Table 1) is that portion where the *p*H is between 9 and 10. For this reason we have chosen the first 800 min of the experiment to calculate the enhancement factor. This portion of the experiment is shown in Fig. 4 and the data used to calculate an enhancement factor were:

Chemical enhancement factors calculated for the first 800 min of the simulation experiment; z and D/z values are averages over the 800 min.

z (µ)	D/z CO_2 (*m/day*)	CO_2 Flux (moles/m²·day $\times 10^{-3}$)		Chemical enhancement
		(*calc*)	(*mean*)	
260	.63	6.94	18.4	2.75

Using the enhancement factor of 2.75 we obtain an invasion rate for CO_2 of 17 ± 8 mmoles/m² day.

Because the chemical properties of lake 227 water are continuously monitored, it has been possible to determine the gas flux for CO_2 independently. Schindler et al. (1972) using a mass balance argument showed that during the period of August 4–18, 1970, the amount of CO_2 invasion required to account for the carbon loss from the epilimnion by sedimentation is 16 ± 4 mmoles/m² day. We believe that the agreement between the CO_2 invasion rate determined in this manner and that calculated using the radon method is good considering the uncertainties in the radon calculations.

O_2 Gas-Exchange Rate

From the carbon sedimentation data it is possible to estimate the gas exchange rate for oxygen and thus the average O_2 partial pressure during August 1970. If lake 227 were abiotic the O_2 partial pressure would be the atmospheric equilibrium value of 266 µmoles/liter. With the addition of algae and bacteria, O_2 is created by photosynthesis and used up by respiration and decomposition. Some of the carbon created during photosynthesis, however, is not reoxidized before it falls through the thermocline of the lake. Considering the approximate equation

$$CO_2 + H_2O \longrightarrow CH_2O + O_2 \qquad (10)$$

every mole of carbon which is sedimented from the epilimnion of the lake leaves behind 1 mole of O_2. If this process is the dominant one controlling the O_2 partial pressure in the lake, the amount of carbon sedimented per day (or, in this case, the amount of CO_2 invasion per day) should be a reasonable estimate of the amount of oxygen escaping from the epilimnion to the atmosphere per day. By this reasoning, the O_2 evasion rate is approximately 17 mmoles/m_2 day, which requires an average O_2 partial pressure of 290 µmoles/liter or approx-

imately 9 mg/liter during August 1970. This partial pressure corresponds to a mean degree of supersaturation of 1.09.

Discussion

We have shown that the gas-exchange rate for CO_2 determined by the radon method is in reasonably good agreement with that determined independently by total carbon balance for lake 227. Although there is no apparent reason to doubt the accuracy of our experiment for determining the chemical enhancement factor, it is not possible to prove whether or not a simulation of this type reproduces the actual gas-exchange conditions for the lake. If, as we believe, the simulation experiment does give a good estimate of the chemical enhancement factor for lake 227, then our best estimate of the stagnant boundary layer thickness ($z \simeq 300$ μ) is fairly good. A chemical enhancement factor of 2.75 and a stagnant boundary layer thickness of 310 μ would predict a CO_2 invasion rate equal to 16 mmoles/m^2 day, i.e. that determined by the carbon budget.

The stagnant boundary layer thickness observed here is greater than those observed for the ocean. Broecker and Peng (1971) found a boundary layer thickness equal to 70 μ ($D/z = 1.8$ m/day) by measuring the evasion of radon in the Atlantic Ocean east of Barbados in May 1969. Munnich and Roether (1967), using bomb ^{14}C as a tracer, found a similar value by measuring the exchange of CO_2 between the ocean and the atmosphere in the North Atlantic. On the other hand, the stagnant boundary layer thickness observed here is of the same magnitude as the largest values observed for the Great Basin lakes by Thurber and Broecker (1970) (see Table 3).

Gas-exchange rates and boundary layer thickness have been correlated with wind velocity (Kanwisher 1963; Hoover and Berkshire 1969; and Thurber and Broecker 1970). Laboratory tests by these authors show that, as expected, with increasing wind speed (in the range of 1.5–13 m/sec) the boundary layer becomes thinner and hence the gas-exchange rate increases. Recently T.-H. Peng (personal communication) has measured the stagnant boundary layer thickness in the central North Pacific in winter. His results indicate that the laboratory experiments are similar to that observed in the ocean. At times of high wind in the winter North Pacific the oceanic stagnant boundary layer is several times thinner than that reported by Broecker and him (1970) in the calmer summer North Atlantic.

The laboratory studies of Hoover and Berkshire (1969) and Thurber and Broecker (1970) indicate a stagnant boundary layer thickness of between 400 and 700 μ for wind speeds equal to or less than 1.5 m/sec. Interestingly, there is no apparent correlation of wind velocity and stagnant boundary layer thickness at these low wind speeds. The wind velocities recorded at the ELA during the period of August 1970 are from 0.5 m/sec to 1.5 m/sec. The anemometer from which these readings were taken is not on lake 227, but rather at a less sheltered, higher location approximately 3 miles from the lake. For this reason the wind speeds may be considered maximum values. Hence the average wind speed at lake 227 is certainly less than 1.5 m/sec suggesting that low wind velocity is the reason for the relatively slow gas-exchange rate.

Another possible controlling factor for gas-exchange is the presence of organic material dissolved in or on the water surface. This possibility has been discussed briefly (Thurber and Broecker 1970). One might think this possibility to be especially relevant to this study because lake 227 has a high standing crop of algae and a thicker than usual film of organic material is conceivable. Recently Pardi (1972) studied the effect of organic substances on the rate of gas exchange through thin nonturbulent films of water. He concluded that to the extent that the organic material decreases evaporation, which cools the surface of the water and creates convection within the film, it decreases gas exchange. He esti-

TABLE 3. Stagnant boundary layer thickness (z) values for lakes and the ocean. (All values except that for lake 227 are taken from Thurber and Broecker 1970, Table 6. D/z values were converted to z values using Thurber and Broecker's diffusion constants for CO_2; $D = 2 \times 10^{-5}$ cm^2/sec at 25 C for the ocean and $D = 1.2 \times 10^{-5}$ cm^2/sec at 10 C for the lakes.)

Water body	z (μ)		
	Maximum estimate	Minimum estimate	Best estimate
Ocean	71[a]		
	90[b]	20[b]	30[b]
Great Salt Lake	377[c]	47[c]	62[c]
Pyramid Lake	122[c]	38[c]	122[c]
Walker Lake	94[c]	38[c]	54[c]
Mono Lake	>187[c]	34[c]	>187[c]
Lake 227	780	195	300

[a]Broecker and Peng 1971 (tropical North Atlantic).
[b]Munnich and Roether 1967 (North Atlantic Ocean; here the minimum is for high latitudes and the maximum is for the tropical ocean).
[c]Thurber and Broecker 1970 (the ranges here are limits of uncertainty).

mates that this effect is only of the order of a 15% reduction but that it may be more important than wind at low wind velocities.

Our observed stagnant boundary layer thickness of 300 μ (with an uncertainty of +300 μ and −100 μ) falls below or in the lower range of laboratory predictions for the wind speeds recorded at the ELA. (The diffusive gas-exchange rate is, thus, in the predicted range or greater.) It is apparent, then, that no mechanism other than slow wind speed need be called upon to explain the relatively low gas-exchange rate observed in lake 227.

Acknowledgments

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Distribution and Uptake of Artificially Introduced Radium-226 in a Small Lake[1]

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Radium-226 was added to lake 227 of the Experimental Lakes Area in August 1970 so that gas-exchange rates between the atmosphere and the lake could be traced by its gaseous daughter product radon-222. Although we expected the radium to remain in solution it did so for only about 1 month. An investigation to locate the radium after it left solution revealed that it was taken up on the bottom in the littoral zone. Further experiments indicated that it had adsorbed to the algal detrital material on the littoral sediment surface. Seasonal movement of this material could thus be monitored by following the movement of the radium with time.

EMERSON, S., AND R. HESSLEIN. 1973. Distribution and uptake of artificially introduced radium-226 in a small lake. J. Fish. Res. Board Can. 30: 1485–1490.

Du radium-226 a été introduit dans le lac 227 de la région des lacs expérimentaux en août 1970, de sorte qu'il a été possible de suivre les taux d'échanges gazeux entre l'atmosphère et le lac par son dérivé gazeux, le radon-222. Bien que nous nous attendions que le radium ait demeuré en solution, ceci n'a duré qu'environ un mois seulement. Des recherches en vue de localiser le radium après sa disparition de la solution ont révélé qu'il avait été absorbé le fond, dans la zone littorale. Des essais plus poussés ont démontré qu'il s'était adsorbé sur les détritus d'algues, à la surface du sédiment du littoral. Il a donc été possible de suivre de façon continue les mouvements saisonniers de ce matériel en observant les mouvements du radium en fonction du temps.

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LAKE 227 is a small dilute lake (area = 5×10^4 m^2, maximum depth = 10 m, conductivity = 20 μmho/cm) of the Experimental Lakes Area (ELA). The lake has been the object of an eutrophication study for the last 4 years. (For a description of the lake see Brunskill and Schindler 1971, and Armstrong and Schindler 1971.) One aspect of the study was an experiment to determine the gas-exchange rate between the atmosphere and the lake by the radon method. To do this 9.7 mc of radium-226 were added to the lake in August of 1970. Radium-226 (half-life = 1600 years) decays to a gaseous daughter product radon-222 (half-life = 3.85 days) which is useful as a tracer in gas-exchange studies. (For details of the gas-exchange experiment and the theory, see Emerson et al. 1973.) The radium spike (added as soluble $RaBr_2$) was enough to bring the epilimnion activity of the isotope in solution to about 200 dpm/liter. (Natural radon and radium activities, measured before the radium addition, were less than 1 dpm/liter at all levels.) Approximately 1 month after the spike radon and radium analysis of the lake water indicated that the radium had not remained in solution. The purpose of this paper is to present the results of our studies, to locate the radium, and to determine the means by which it was removed from solution.

To familiarize the reader with the problem, radon and radium activities of the lake water from the time of the spike, August 1970, to June 1971 are presented here (Fig. 1). The radium data of August 1970 (Fig. 1b) are lower limits because of a sample storage problem (see Emerson et al. 1973); nevertheless, by September 1970, when the problem was corrected, the total radium activity was about an order of magnitude less than that which would be expected from the amount added. The radon activity, on the other hand, was between 5 and 10 times that of the dissolved radium in September 1970, and through May 1972 remained between 36 and 71% of what would be expected for secular

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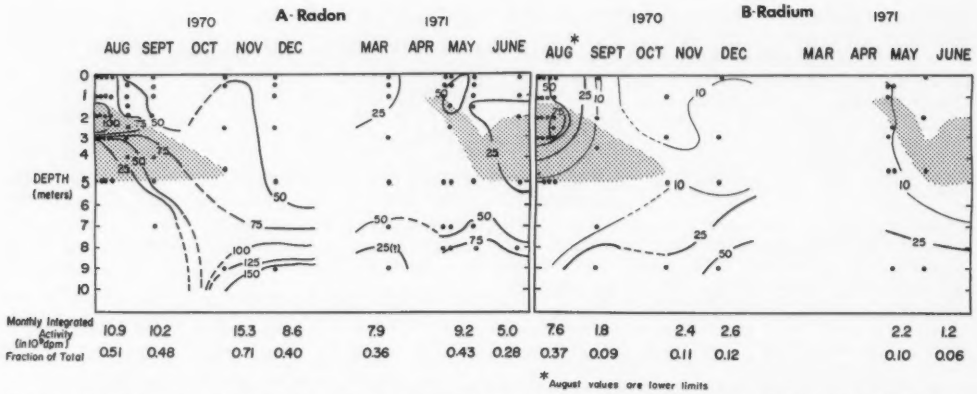
A-Radon
B-Radium
1970
1971
AUG SEPT OCT NOV DEC MAR APR MAY JUNE
AUG* SEPT OCT NOV DEC MAR APR MAY JUNE
DEPTH (meters)
Monthly Integrated Activity (in 10¹⁰ dpm)
10.9 10.2 15.3 8.6 7.9 9.2 5.0
7.6 1.8 2.4 2.6 2.2 1.2
Fraction of Total
0.51 0.48 0.71 0.40 0.36 0.43 0.28
0.37 0.09 0.11 0.12 0.10 0.06
*August values are lower limits

FIG. 1. Isopleth maps of radon (A) and of radium (B) activity (in dpm/liter) from August 1970 to June 1972. Dots indicate sample locations. The stippled area is the portion of the lake water which has a temperature gradient greater than 2 C/m. Monthly radon and radium activities integrated throughout the whole lake volume and the fraction of the total added are also given ($2{:}14 \times 10^{10}$ dpm radium was added to the lake on August 2, 1970).

equilibrium with all the added radium. Nearly all this radon resided in the epilimnion of the lake until fall 1970.

Apparently the radium, after it left solution and before fall turnover, was located in an area from which radon, created upon radium decay, could return to the epilimnion of the lake. This suggested the surface sediment in the littoral zone as the source region during late August and September of 1970.

Methods

Radium Distribution

To confirm the hypothesis that radon was diffusing from the littoral zone, radon and radium analysis was done along transects in the lake and the radium activity of algal and detrital material of the littoral region was measured. These investigations were carried out in the summer of 1971, about 1 year after radium was added to the lake.

Seven water samples at a depth of ½ m were taken on each of two perpendicular transects in July 1971. The sampling methods for lake water are given in Emerson et al. (1973). Briefly, the samples were collected in 2-liter evacuated glass bottles which were opened and allowed to fill with water from a depth of ½ m. The radon and radium analysis methods are those of Broecker (1965). To determine the radium activity of the littoral region, surface sediment and algal and detrital material which had been removed from littoral rocks were analyzed for their radium content. The radium activity of surface sediment was determined by coring the sediment, replacing the lake water above the sediment–water interface with distilled water, and analyzing the distilled water for radon after it remained on the sediment for about 1 week. The amount of radon which had diffused into the distilled water gave a rough estimate of the amount of radium in the surface sediment. The radium activity of algal and detrital material on the rocks was determined by either scrubbing the rocks with a brush to remove the material, or where algal growth was thick, scraping the rocks to remove it.

Since the epilithophyton algal community which grows on the rocks consists largely of diatoms, the algal–detrital material which had been scraped from the rocks was subjected to separation of the hard and soft parts. For a description of the epilithophyton of lake 227 and the method of separation of the organic and silicious material see Stockner and Armstrong (1971). The two fractions were analyzed for their radium content.

To compare the above results with the situation in nonlabelled lakes the same procedures were followed with littoral rocks from lakes 239 and 240 which have natural radium activities (about 0.1 dpm/liter). For a description of these lakes see Brunskill and Schindler (1971).

In 1972 several deep water surface sediment samples were taken. They were collected by lowering pressure tubing to the sediment–water interface and attaching the tubing to an evacuated bottle, thus allowing the bottle to fill with lake water and surface sediment. After the lake water was decanted from the sample, the sediment was dried and weighed. Acidified distilled water was then added to liberate any bound radium, and radon analysis was done on the water after 1 week. Unfortunately with these samples we are not able to put the results in units of radium activity per area of the lake bottom because it was impossible to determine what fraction of the sediment was from the bottom surface. The results of these samples are interpreted qualitatively.

Method and Rate of Radium Uptake

Because the results of the previous experiments indicated that radium was highly concentrated in rock scrapings, we attempted to correlate radium uptake with

carbon fixation of the epilithophyton. Several algal–detrital covered rocks from the littoral zone of lake 239 were enclosed with lake water in a plexiglass "Benthic Chamber" and placed on the bottom of the lake so that light intensity for the algae would be the same as that in the natural situation. Carbon fixation was determined by CO_2 and O_2 measurements over a 4-hr period. (For details of the benthic production experiments see Schindler et al. 1973.) Radium labelled water was injected into the chamber at the beginning of the experiment and radium analysis was made on the water 4–5 hr later. This experiment was performed on natural lake water as well as on water which had the following additions: (1) 4% Formalin to poison the algae; (2) additional nutrients nitrogen and phosphorus to increase production; and (3) barium ion to determine the effect on radium uptake of high concentrations of a chemically similar element.

To determine the rate of radium uptake algal–detrital material of lake 239 was scraped from the littoral rocks, wet volume measured, and placed in a 300-ml beaker. The suspension was stirred with a magnetic stirrer and radium-labelled distilled water was added. In one case the algal–detrital material was boiled for 10 min before radium labelling so that the algae would be killed. Samples (5 cm^3 each) were removed for radium analysis by a glass syringe through a Swinnex 0.45-μ millipore filter. Radium analysis on these samples was done by liquid scintillation counting in a dioxane fluor. [To avoid confusion between the radium activity and the activity of its long-lived daughter products Pb210, Bi210, and Po210, the radium solution was cleaned by passage through a Dowex 1-X anion exchange column before being added to the algal–detrital suspension.] The 5 cm^3 samples were injected directly into scintillation vials and bubbled with helium to remove radon gas. Three hours were allowed before counting so that the short-lived randon daughter products would decay to negligible activities. Calibrations have shown that counting efficiencies for this procedure are near 100% (R. Hesslein unpublished data).

Results

Radium Distribution

From the horizontal transects of lake 227 (Fig. 2) it is clear that there was a decrease in the activity of radon toward the center of the lake. These results support the assumption that the radon source was the littoral zone.

The radium activity of rock scrapings and surface sediment taken in 1971 from lakes 227, 239, and 240 (Table 1) further indicate that radium was concentrated on the littoral bottom surface. The results of two successive scrapings of the same rocks from lake 227 show that indeed the radium analyzed in these scrapings was concentrated in the algal or detrital coating of the rock and not from the rock itself. From an estimate of the exposed surface

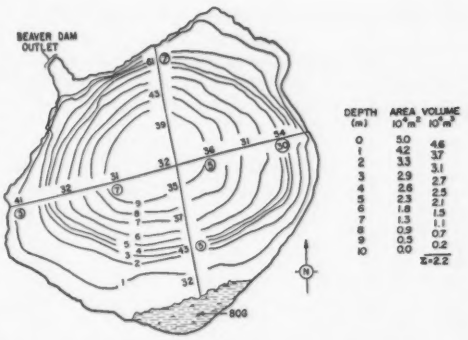

FIG. 2. Horizontal transects showing radium and radon activities for lake 227 in dpm/liter (all samples taken at ½ m; circled numbers are radium values). The bathymetric chart is from Brunskill and Schindler (1971).

area of the rocks scraped, between 15 and 50 dpm radium/cm^2 existed there as compared to between 1 and 2 dpm/cm^2 on the surface sediment. Apparently the radium was taken up preferentially on the rock surfaces of the littoral zone. A rough estimate of the area of the lake bottom between 1 and 4 m is 2×10^8 cm^2. Since 2.1×10^{10} dpm radium was added to the lake the average activity per unit area in the littoral zone should be approximately 100 dpm/cm^2. This value is two to five times that found on the rock surfaces which cover only a fraction of the lake bottom and 100 times that on the littoral sediment surface. Since these measurements were made about 1 year after the radium became adsorbed, movement of bound radium with time in the lake may account for the discrepancy between calculated and observed activities.

Littoral rock scrapings from lakes 239 and 240 indicated that the radium in natural concentrations was also taken up by algal-detrital coatings. In both cases (natural and artificial radium concentrations) the organic fraction had nearly all of the radium associated with the scrapings; the siliceous hard parts of the algae and any detrital silicates had retained negligible amounts of radium after the separation procedure.

Measurements of the radium activity of lake 227 sediment in the summer of 1972 (1 year after the above measurements and 2 years after the lake was labelled) showed activities in the surface sediments below 8 m to be as high as 660 dpm/g of dried sediment. The activity per unit area in these samples is impossible to determine, however, the shear magnitude of the activity indicates that radium during the years 1970 and 1971 had moved from littoral to profundal areas of the lake.

Method and Rate of Radium Uptake

In all experiments using the plexiglass chambers the fraction of radium removed from the water was between 47 and 62% over a period of 4–5 hr.

Chamber	Initial Ra activity (*dpm*)	Final Ra activity (*dpm*)	% Ra removed
Ra only	6020	2660	56
Ra + Ba 10^3 × Ra	5600	2380	57
Ra + Ba 10^6 × Ra	6160	2830	54
Ra + Ba 10^9 × Ra	10,080	4380	60
Ra + Formalin 4%	11,020	4200	62
Ra + P Overdose	10,800	5690	47

There was no relation to the carbon fixation rate. The Formalin-killed chamber and a chamber accidently killed by phosphorus overdose show the same 47–62% radium removal as the control chamber. Concentrations of barium of 10^3, 10^6, and 10^9 (3.3×10^{-6}, 3.3×10^{-3}, and 3.3 g/liter) times the concentration of radium added also had no effect on the amount of radium removed. A chamber blank showed that a negligible amount of radium was taken out of solution by adsorption to the chamber walls.

The result of the experiments to determine the rate of radium removal (Fig. 3) indicated that there was no significant difference between the rate of removal by unaltered algal–detrital material and algal–detrital material which had been boiled. The algae–detritus used in these experiments were nearly equivalent in wet volume, hence they probably varied by less than 30% in dry weight. The

Table 1. Radium activity of littoral rock scrapings and sediment from lakes 227, 239, and 240.

Date	Lake	Size and description of sample	Total radium (*dpm*)	Radium in organic soft parts (*dpm*)	Radium in siliceous hard parts (*dpm*)	Radium/cm² lake bottom surf. (*dpm/cm²*)
Aug. 1971	227	Scrapings of seven rocks from the littoral zone	3950			48
	227	Second scraping of above rocks	184			2.2
	227	Scrapings of eight rocks from the littoral zone	1070			13
	227	31 cm² of sediment-water interface at ½ m	40			1.3
	227	31 cm² of sediment-water interface at 4 m (below thermocline)	5			.2
	227[b]	50 g wet weight[a] of algae scraped from littoral rocks	3340	3342	8	
	239[b]	50 g wet weight[a] of algae scraped from littoral rocks	30	30	0	
July 1972	239[b]	.28 g dry weight of algae scraped from littoral rocks	2.6			
	240[b]	.21 g dry weight of algae scraped from littoral rocks	4.8			
	240[b]	.27 g dry weight of algae scraped from littoral rocks	3.6			
	240[b]	.26 g dry weight of algae scraped from littoral rocks	1.2			
	227[b]	5.12 g dry weight lake sediment from 7 m	143			
	227[b]	6.12 g dry weight lake sediment from 8.5 m	810			
	227[b]	.46 g dry weight lake sediment from 9.3 m	302			
	227[b]	1.1 g dry weight lake sediment from 9.3 m	390			

[a]Wet weight is the total weight of algae and water after as much water as possible was decanted from the suspension; we estimate water accounts for 80–90% of the weight.

[b]The radium activity per unit area for algae scraped from rocks, and for deep bottom sediments is uncertain (see text).

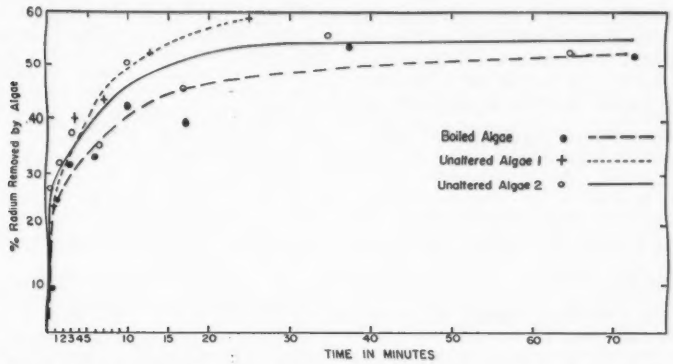

FIG. 3. Percent radium removed from solution by algae scraped from lake 239 littoral rocks as a function of time.

time for the activity of radium removed to reach one half its final value was 1–4 min. The accuracy of the sampling time was ±20 sec.

Discussion

Since the radium is more heavily concentrated on rock surfaces than on the sediment surface we suspect that the epilithophyton algae which inhabit the rocks in the littoral zone were more important as a radium sorption agent than detrital material which should be evenly distributed on the sediment and rocks. Because of the paucity of the data it is not possible to determine more precisely the roles played by the detrital and algal material in radium uptake.

Although the relatively high activity of radium on the rock surfaces indicates that algae play a primary role in the sorption of radium, there was no correlation between photosynthesis and radium uptake in the plexiglass chamber and rate experiments. In all cases the uptake of radium was the same whether the algae were alive or dead. These results along with the fact that no radium existed in the siliceous hard parts of the algae indicate that the algal material removes the isotope from solution by adsorption rather than incorporation during photosynthesis. The rapid rate at which the radium was removed from solution and the lack of any decrease in uptake by diluting it with 10^9 times its concentration of a chemically similar element indicate that the method of removal is either highly specific or has a very large capacity.

The uptake of radium by aquatic organisms has undergone considerable investigation in the past decade (Popova 1965; Parasant 1967; Holtzman 1969; Havlik 1971). These investigations have been made largely because of contamination of natural waters by this nuclide during uranium exploration. Havlik (1971) performed radium uptake experiments on cultured algae which showed that in some species the isotope was absorbed and metabolized but that the main method of uptake was adsorption. Although the species cultured by Havlik were not those present in our lakes, his work lends support to the method of radium uptake indicated by our experiments.

It is safe to assume that the radium remained adsorbed for a period of 10 months (August 1970–May 1971) because the integrated radon content of the lake remained between 8 and 15 × 10^9 dpm and very little radium was in solution during that period. Although it remained bound, the radium became redistributed in the lake with time. This is evident from the radon data, which indicate a redistribution of its source from the littoral regions in summer 1970 to deeper in the lake after September. Redistribution is confirmed by very high radium activity of the sediment taken from the lake bottom in 1972 (Table 1).

If the radium is bound to the algal–detrital material, its distribution in the lake is useful as a tracer for following the movement of littoral sediment with time. Hence, some of the algal–detrital sediment is transported from its summer 1970 littoral position to a more homogeneous distribution in the lake following fall turnover in October. This explanation for the redistribution of radium is supported by Stockner's (1971) observation that littoral diatom species are consistently found in the deep sediment of the ELA lakes.

The redistribution and redeposition of littoral sediment in a Michigan lake was studied by Davis (1968). She observed that during times of overturn

the shallow water pollen of the lake was stirred and redeposited over the entire lake basin. The net result is the movement of material from shallow to deep surfaces of the lake. This method of redistribution of littoral bottom material is probably the explanation for the relocation of radium and hence littoral sediments with time in lake 227.

The movement of the radium with time after it has left solution is more easily understood than the method by which it is initially deposited in the littoral zone. The individual roles of planktonic and benthic algae and detrital material in radium uptake are not well understood. These uncertainties along with the rate-controlling aspects of radium uptake await further explanation.

Acknowledgments

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Vertical Eddy Diffusion Studies in the Thermocline of a Small Stratified Lake[1]

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HESSLEIN, R., AND P. QUAY. 1973. Vertical eddy diffusion studies in the thermocline of a small stratified lake. J. Fish. Res. Board Can. 30: 1491–1500.

Values for K, the coefficient of vertical eddy diffusion in the thermocline of lake 227, a small, highly stratified, and artificially eutrophied lake in northwestern Ontario, Canada, are presented. Three methods: the classical temperature method of McEwen, a dye method, and a new method using a RAGAPOS (Radon Gas Point Source), were used. Results ranged from 2×10^{-4} cm²/sec by the Rn method to 34×10^{-4} cm²/sec by temperature method; the dye method resulted in an average value of 12×10^{-4} cm²/sec. The recycling of ΣCO_2 resulting from particulate degradation below the epilimnion was calculated. The results ranged from 8 to 53% of the net loss of carbon from the empilinion depending on the K value used. Results from the radon method are considered to be more accurate than results from the temperature method.

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Nous donnons les valeurs de K, coefficient de diffusion tourbillonnaire verticale, dans la thermocline du lac 227, un petit lac hautement stratifié et artificiellement eutrophié du nord-ouest de l'Ontario, Canada. Nous avons employé trois méthodes: la méthode classique des températures de McEwen, une méthode par colorants et une nouvelle méthode basée sur un RAGAPOS (Radon Gas Point Source). Les résultats s'échelonnent de 2×10^{-4} cm²/sec avec la méthode du Rn à 34×10^{-4} cm²/sec avec la méthode des températures; la méthode des colorants donne une valeur moyenne de 12×10^{-4} cm²/sec. Nous avons calculé le recyclage de ΣCO_2 résultant de la dégradation particulaire au-dessous de l'épilimnion. Les résultats varient de 8 à 53% de la perte nette du carbone de l'épilimnion selon la valeur de K utilisée. Nous sommes d'avis que la méthode du radon donne des résultats plus précis que la méthode des températures.

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THE processes responsible for eutrophication cannot be accurately understood without knowledge of the rates of recycling of important nutrients removed from the euphotic zone by particulate flux. The return to the euphotic zone by diffusion of the chemical species resulting from the degradation of the particulates can be calculated using the Fick's Law relationship Flux $= K\frac{dc}{dz}$, where K is the coefficient of vertical eddy diffusion and $\frac{dc}{dz}$ is the vertical gradient of concentration of the chemical species. The purpose of the experiments described in this paper was to determine the vertical eddy diffusion and to calculate the flux of CO_2 in the thermocline of lake 227. Lake 227 is a small, well sheltered, circular lake, approximately 5×10^4 m² in area with a maximum depth of 10 m (Fig. 2 Emerson and Hesslein 1973), located in the Experimental Lakes Area (ELA) of northwestern Ontario, described by Brunskill and Schindler (1971). This lake is the site of a long-term eutrophication study by the Freshwater Institute group (Schindler 1971, Schindler et al. 1971, 1973). In addition to the classical temperature method of McEwen (1941), a method using Rhodamine B dye and a new method using the RAGAPOS (Radon Gas Point Source) were used to determine K. The aim in using the two additional methods was to get results from methods which "remembered" mixing over different periods, to establish the usefulness of the radon method, which involves only a single set of measurements and reflects mixing over a short time period (6 days), and to

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check the validity of results from Hutchinson's method. Two innovations were required for the study, the limnocorral and the RAGAPOS; these are described below. All the dye and the radon experiments were performed in the limnocorral in lake 227 during July, 1972. The temperature data used for Hutchinson's method were for the whole lake, taken in 1971.

New Experimental Techniques

The limnocorral used in these experiments was a triangular enclosure suspended by surface floats and anchored in position at three corners, (Fig. 1A). It was made of a sheet of yellow vinyl impregnated fabric 14.6 m (48 ft) long and 6 m deep with a plastic interlocking zipper allowing closure of the triangular area. There was a chain sewn along the bottom of the limnocorral for weight and styrofoam beads in a 15-cm diameter flotation hem on the top edge. Grommets were provided for attaching anchoring ropes. The limnocorral was manufactured by Kemper Plastics of Torrance, California.

The function of the limnocorral was to separate an area of the lake large enough to approximate lake conditions (see Discussion) but small enough to allow use of safe and inexpensive amounts of tracers at easily measurable concentrations. The limnocorral allows many experiments to be performed without affecting the condition of the whole lake. It can be redeployed at any time, allowing the initiation of new experiments in unaltered lake water. For epilimnetic studies the limnocorral is best suited for lakes with relatively shallow and steep thermoclines as the thermocline will then serve as an effective "bottom" to loss of materials in solution.

The radon gas experiment required a constant flux of Rn^{222} to the surroundings with as little loss as possible of the parent radionuclide Ra^{226} (an aqueous solution of $RaBr_2$). Rn^{222} is a radioactive gas with a half-life of about 4 days. The RAGAPOS was designed for this purpose, (Fig. 1B). It is a 1-cm^3 well drilled in a PVC rod and covered with a gas permeable membrane, General Electric's MEM-213. A cap secured over the membrane makes the well watertight. Laboratory experiments showed that in a 1-week period approximately 90% of the radon produced escaped and $< 1\%$ of the radium in the well was lost. One microcurie, 2.2×10^6 dpm, of radium was used as a source concentration. The $RaBr_2$ solution was absorbed onto a small piece of sponge glued to the bottom of the well.

Temperature Method

To apply McEwen's (1929) method as used by Hutchinson (1941), a series of weekly temperature profiles were needed throughout the heating period. Thirteen profiles were taken in the middle of the lake from May 17 to August 10, 1971 at 1-m intervals, over the depths 3–8 m, using a Whitney model TC-5A thermistor, with an accuracy of ± 0.1 C.

To estimate the vertical eddy diffusion by the temperature method, one assumes that the transmission of heat through the "clinolimnion" is by vertical diffusion only. The "clinolimnion" is defined by Hutchinson as

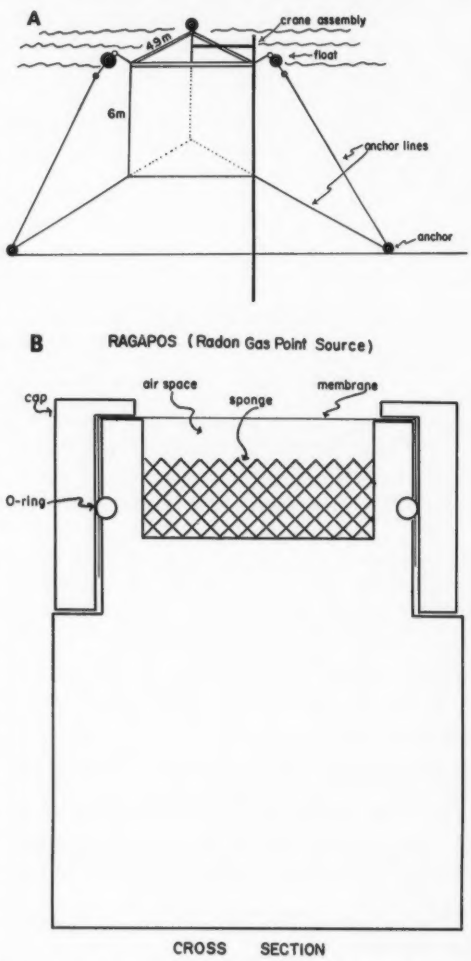

FIG. 1. A, Limnocorral; B, Radon Gas Point Source (RAGAPOS).

the linear portion of the plot of log $\frac{d\theta}{dt}$ vs. z curve shown in Fig. 2, where θ = temperature, t = time, and z = depth. A least squares regression program used the average temperature over the heating period at each depth to calculate the constants C, a, and C_1 where C represents the apparent temperature of overturn for the lake. Using these empirically determined constants and the best fit slope of $\frac{\Delta\theta}{\Delta t}$ at each depth shown in Fig. 3, one could calculate the apparent eddy diffusion coefficient, K, at each depth, summarized in Table 1. Values of θ–C vs. z, and $\frac{\Delta\theta}{\Delta t}$ vs. z can then be calculated from the relationships $\theta - C = C_1 e^{-az}$ and $\frac{d\theta}{dt} = KC_1 a^2 e^{-az}$, respectively. These values are plotted and the fit to the observed θ–C vs. z and $\frac{d\theta}{dt}$ vs. z are shown in Fig. 2. The accuracy of these fits are Hutchinson's "criteria of validity." A more detailed explanation of this method can be found in Hutchinson (1941).

The results obtained gave an average K = 33×10^{-4} cm²/sec in the thermocline, representing an average eddy diffusion coefficient over the 12-week period, May 17–August 10. As mentioned this method assumes there is no other heat production or loss in the clinolimnion region. The possible addition of heat from solar radiation, biological activity, and geothermal heating were calculated. Following Bachmann and Goldman (1965) and using 2.1/m as an average coefficient of extinction for all wave lengths, it was found that the percentage solar heating contribution decreased as expected from 3 m (15%) to 6 m (1%). The biological heating calculated from heat of formation of CO_2 from simple organics and amount of CO_2 produced in 1-m layers in the thermocline (see Discussion) resulted in an increasing percentage addition from 3 m (5%) to 6 m (16%). The geothermal heat addition using an approximation of 1.5 μcal/cm² sec was found to be negligible. The combined effects of solar radiation and biological heat were found to contribute approximately 15–20% of the total temperature increase per month at the 3-m, 4-m, 5-m, and 6-m levels. Considering these calculations, the $\frac{d\theta}{dt}$, and therefore the K value, should be lowered by 15–20%. This would result in a corrected average K value of about 27×10^{-4} cm²/sec.

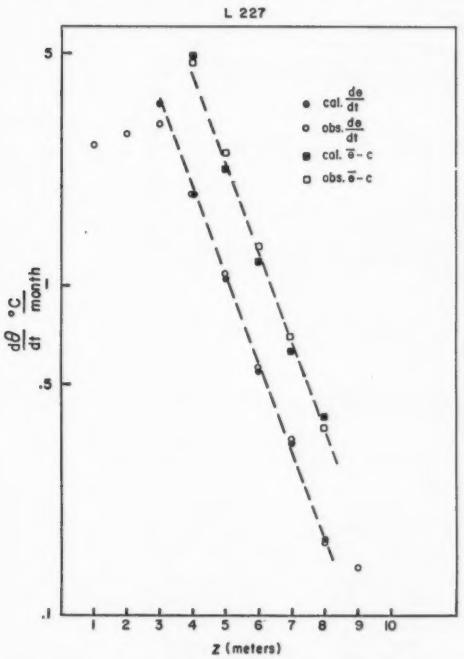

FIG. 2. Observed $\frac{d\theta}{dt}$ and θ–C vs. depth relationship in lake 227 over the heating period May 17–August 10, 1971 and those calculated by McEwen's method (see text).

Dye Method

Analyses for Rhodamine B dye were made with a Turner model 111 fluorometer, sensitive to concentrations of 10^{-8} g/ml ± 3%. The injection of dye was made by pouring a solution of Rhodamine B, 3 g in 100 ml of lake water, down a rubber tube with a T connector at its bottom end. The tube was weighted to keep it vertical and the top was held 50 cm above the lake level to allow a slow flow of the mixture. The tube was then flushed with an additional 200 ml of lake water. Sampling was done with a peristaltic pump through a hose with a T connector and suspended from a crane assembly, which allowed 100 ml samples to be taken from the middle of the limnocorral. Samples were taken at 10-cm depth intervals with accuracy to better than ± 1.5 cm. At each new depth the hose was allowed to clear of water from the previous depth.

The dye was injected in the thermocline and was assumed to be well mixed horizontally due to the lack of thermal stratification in the horizontal direction. This assumption was shown to be correct by the equality of the integrated amount of dye calculated from the center profile and the amount of dye initially injected. This condition of horizontal homogeneity allows the application of the one dimensional diffusion equation:

$$\frac{dc}{dt} = K \frac{d^2c}{dz^2}$$

By measuring the concentration at a distance z from the injection depth over a period of time, a time t will be found when $\left(\frac{dc}{dt}\right) = 0$. When this condition exists,

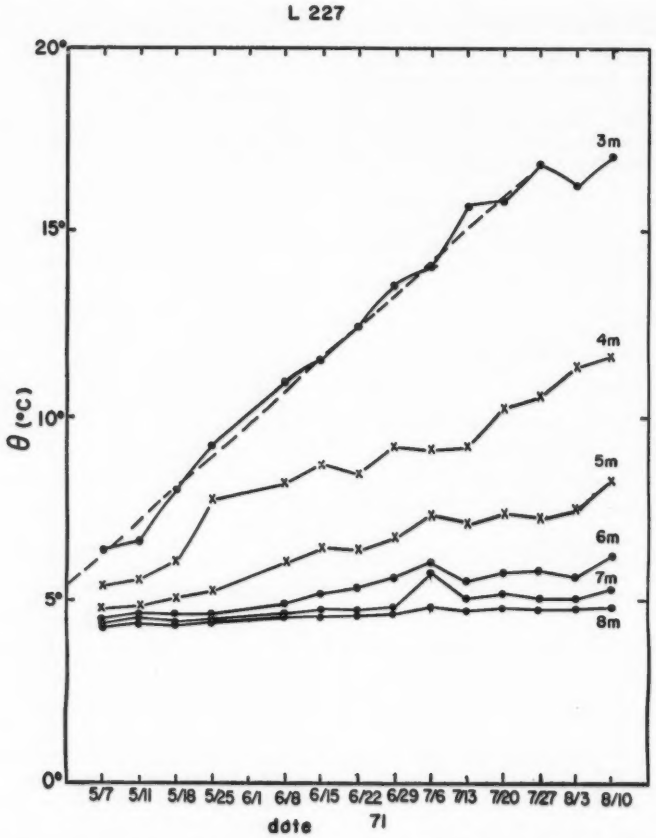

FIG. 3. Temperature variations in lake 227, at 1-m intervals in the clinolimnion, during the heating period May 17–August 10, 1971.

TABLE 1. Vertical eddy diffusion coefficients (K*) calculated by McEwen's heat method.

z (*m*)	$\bar{\theta}$ (*C*)	$\frac{d\theta}{dt}$ (*C/month*)	K** (m^2/day)	K** (cm^2/sec)
3	13.20	3.07	28.7×10^{-3}	33.3×10^{-4}
4	9.12	1.87	27.9×10^{-3}	32.3×10^{-4}
5	6.64	1.08	29.5×10^{-3}	34.2×10^{-4}
6	5.36	0.557	29.4×10^{-3}	34.1×10^{-4}
7	4.83	0.340	32.9×10^{-3}	38.2×10^{-4}
8	4.60	0.167	32.8×10^{-3}	38.0×10^{-4}

*μ^2 in Hutchinson (1941).
**Using $C = 4.21$, $a = 0.634$, $C_1 = 59.49$.

$K = \frac{z^2}{2t}$. (See Crank (1956) for a more complete discussion.)

The average K value for diffusion of Rhodamine B dye as calculated by the above method was 12×10^{-4} cm²/sec. The range of values is summarized below:

Z* (*cm*)	*t* (*days*)	K (*cm²/sec*)	K *m²/day*
40 (down)	11.2	12×10^{-4}	10×10^{-3}
50 (down)	11.6	8×10^{-4}	6.9×10^{-3}
60 (down)	11.8	18×10^{-4}	16×10^{-3}
30 (up)	4.8	11×10^{-4}	9.5×10^{-3}

*Distance from peak, which is taken as 3.1 m.

The plots of concentration versus time from which these values were calculated are shown in Fig. 4. The concentration profiles for five of the 10 sampling dates are given in Fig. 5; one should observe the finite thickness to the injection layer. This is not insignificant with respect to the diffusion rate calculated. Considering this, the value of the calculated K should be taken as an upper limit.

Radon Method

Analyses for radon were made by the method described by Broecker (1965). Results from this method are reproducible within 5%. Samples were taken in 500-ml

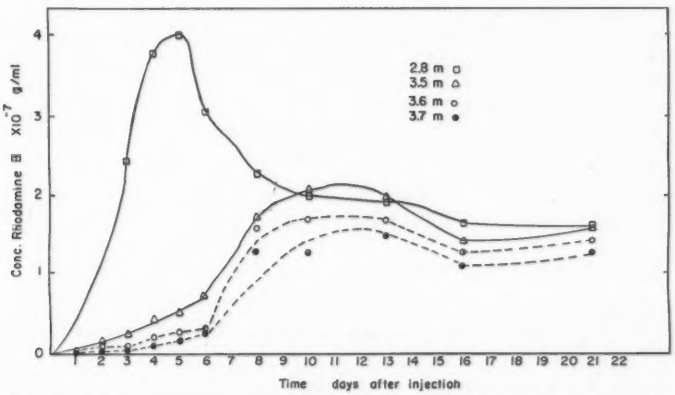

FIG. 4. Variation in the dye concentration at certain depths inside the corral over the 21-day period following the dye injection at 3.1 m.

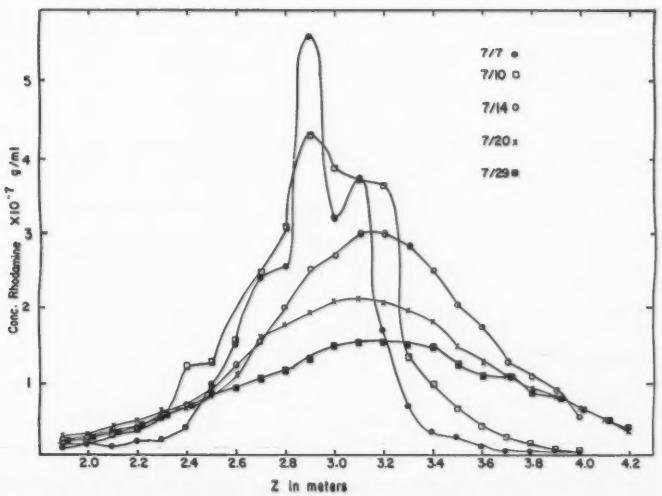

FIG. 5. Dye concentration vs. depth inside th corral on 5 sampling days.

evacuated bottles by the same hose and crane arrangement as for the dye. The hose line was cleared at each depth of water from the previous depth with a peristaltic pump. This was done to avoid excessive raising and lowering of the hose, which might have disturbed the profile. The RAGAPOS was rigidly fixed with respect to the bottom of the lake at a depth of 3.0 m.

The radon method for determining eddy diffusion is based on the principle that a steady state condition will be achieved in which the supply by eddy diffusion of radon atoms to any point distant from the source will be exactly balanced by the radioactive decay of the radon atoms at that point. The concentration of radon at any point will reflect the eddy diffusion history of the previous 6 days, the mean life of a radon atom. Once a steady state condition exists, one measurement of the concentration gradient is sufficient to determine the eddy diffusion coefficient.

One must assume a planar source to apply a one-dimensional diffusion equation. As in the dye experiment this assumption was verified by the agreement of the integrated value of the Rn profile in Fig. 6 with the known flux from the RAGAPOS. To express diffusion of a radioactive substance, a decay term must be added to the one-dimensional diffusion equation so that $K \frac{d^2c}{dz^2} - \lambda C = \frac{dc}{dt}$. In the steady state condition $\frac{dc}{dt} = 0$ so $K \frac{d^2c}{dz^2} = \lambda C$. Following Broecker et al. (1968) the solution to the steady state condition is $C = C_0 e^{-z\sqrt{\lambda/K}}$ where λ is the decay constant (2×10^{-6} sec^{-1} for radon). K can be found by determining a half-distance, $z_{\frac{1}{2}}$, the distance over which the concentration drops by a factor of two. This gives $K = \left(\frac{z_{\frac{1}{2}}}{ln2}\right)^2 \lambda$ which for radon is equal to $4.2 \times 10^{-6} (z_{\frac{1}{2}})^2$ cm^2/sec.

The average K value found by the radon method was 4.8×10^{-4} cm^2/sec. Calculated values of K from the radon experiment are shown below:

$z_{\frac{1}{2}}$ (cm)	K (cm^2/sec)	K (m^2/day)
7	2.03×10^{-4}	1.8×10^{-3}
8	2.7×10^{-4}	2.3×10^{-3}
9	3.4×10^{-4}	2.9×10^{-3}
10	4.2×10^{-4}	3.6×10^{-3}
11	5.1×10^{-4}	4.4×10^{-3}
12	6.1×10^{-4}	5.3×10^{-3}
15	9.5×10^{-4}	8.2×10^{-3}

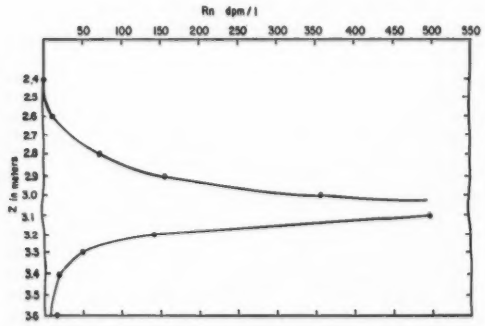

FIG. 6. Radon activity vs. depth, taken 17 days after the RAGAPOS had been placed at 3.0 m inside the corral.

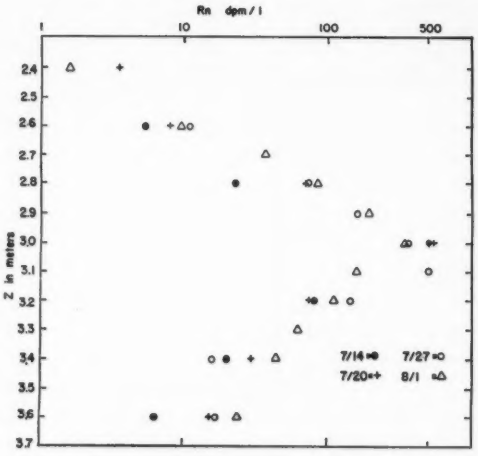

FIG. 7. Log of radon activity versus depth on 4 sampling days. The RAGAPOS was placed inside the corral at 3.0 m, on July 7.

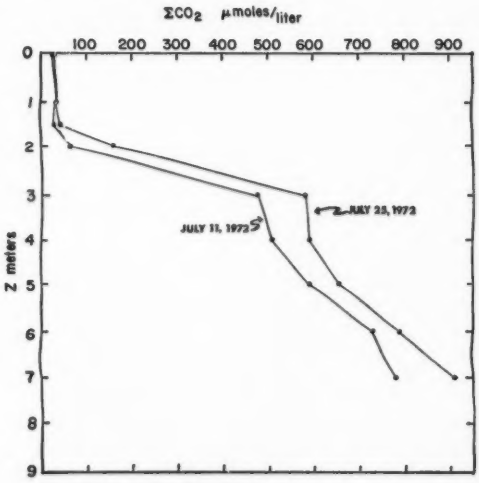

FIG. 8. Total CO_2 [DIC] versus depth in lake 227, on July 11 and July 25, 1972.

Half distances for radon ($z_{\frac{1}{2}}$) from which these K values were calculated and the dates of their measurement follow:

1972		7/14	7/20	7/27	8/1	Average
$z_{\frac{1}{2}}$ (cm)	Up	10	7	8	9	8.5
	Down	11	15	7.5	15	12.1

The value for $z_{\frac{1}{2}}$ were read from plots of log activity of radon vs. z shown in Fig. 7. An example of one profile, taken 17 days after placement of the RAGAPOS is shown in Fig. 6. Because of the spacing of the measurements, about 6 days, the K values for each date represent a value for mixing over a distinct period unaffected by mixing in the previous period.

ΣCO_2 Calculations

Important differences in the portion of the carbon lost from the epilimnion which is returned as ΣCO_2 by mixing are arrived at using the K values determined by the temperature, dye, and radon methods. Profiles of ΣCO_2 are shown in Fig. 8. The flux of ΣCO_2 into the base of the epilimnion is equal to the gradient in the layer just below the epilimnion (2–3 m) multiplied by the vertical eddy diffusion coefficient, K. The total flux was arrived at by multiplying by the area of the lake at 2.5 m (Brunskill and Schindler 1971). These calculations are presented in Table 2. The figure of 625 moles/day as the loss of carbon from the epilimnion is an average late summer value

TABLE 2. ΣCO_2 flux to epilimnion, July 11–25, 1972.

$\frac{dc}{dz}$ (2–3 m) $\frac{\text{mole}}{\text{m}^4}$	K $\frac{\text{m}^2}{\text{day}}$	A(2.5 m) m^2	ΣFlux $\frac{\text{mole}}{\text{day}}$	% of lost carbon
0.415	26×10^{-3}	3.07×10^{4}	330	53%
0.415	10×10^{-3}	3.07×10^{4}	127	20%
0.415	4×10^{-3}	3.07×10^{4}	51	8%

TABLE 3. ΣCO_2 production at 1 m depth intervals, July 11–25, 1972.

K $\frac{\text{m}^2}{\text{day}}$	Z meters	$\frac{dc}{dz}$ $\frac{\text{mole}}{\text{m}^4}$	F $\frac{\text{mole}}{\text{m}^2\ \text{day}}$	ΣF $\frac{\text{mole}}{\text{day}}$	ΔF $\frac{\text{mole}}{\text{day}}$	ΔC $\frac{\text{mole}}{\text{day}}$	ΔC–ΔF $\frac{\text{mole}}{\text{day}}$
	2.5	.415	1.1×10^{-2}	330			
					−314	210	524
	3.5	.023	6.0×10^{-4}	16			
26×10^{-3}					31	150	119
	4.5	.073	1.9×10^{-3}	47			
					28	110	82
	5.5	.136	3.5×10^{-3}	75			
							725
	2.5	.415	4.15×10^{-3}	127			
					−121	210	331
	3.5	.023	2.3×10^{-4}	6.3			
10×10^{-3}					12	150	138
	4.5	.073	7.3×10^{-4}	18			
					11	110	99
	5.5	.136	1.4×10^{-3}	29			
							568
	2.5	.415	1.7×10^{-3}	52			
					−50	210	260
	3.5	.023	9.2×10^{-5}	2.5			
4×10^{-3}					4.6	150	145
	4.5	.073	2.9×10^{-4}	7.1			
					3.9	110	106
	5.5	.136	5.4×10^{-4}	11			
							511

(Schindler et al. 1973). If the K value determined by Hutchinson's method is the true value, more than half the carbon lost from the epilimnion is returned as ΣCO_2, whereas the K from the radon method predicts only 8% return of carbon.

Considerable information about the production of ΣCO_2 at different levels in the thermocline was found by subtracting the net flux ΔF out of a level from the change in concentration of ΣCO_2 in the level. K is assumed to be constant over the depth range of 2–6 m. When K is held constant changes in flux are dependent on only the gradient of concentration ($\text{Flux} = K\frac{dc}{dz}$).

By this method fluxes over the 1-m intervals in the thermocline were calculated and the flux over each interval assumed to best represent the flux at the center of the interval. The total flux was again calculated by multiplying the flux at the center by the area of the lake at that level. ΔF is the difference of total flux into and out of a level (i.e. 4.5 m–5.5 m). ΔC is the rate of change in concentration of ΣCO_2 over the 2-week period July 11–July 25 as calculated from Fig. 8. The calculations are represented in Table 3. It can be seen that there is considerable production of ΣCO_2 by degradation in the thermocline, especially in the region just below the epilimnion.

Discussion

The difference in the resultant K values obtained by the three methods can be explained in two ways. First, the K values calculated by each method are average K's over the period of data collection; for the temperature method this period was 12 weeks, for the dye method it was 3 weeks, and for the radon method the period was about 6 days. Second, the radon and dye experiments performed inside the corral, gave lower estimates of K than the heat method, which was calculated from whole lake data; the presence of the limnocorral could inhibit mixing processes.

Considering the heat method, the use of a one-dimensional heat flux model requires that 1) vertical advection is negligible, 2) there is horizontal thermal homogeneity in each layer, i.e. there is no horizontal temperature gradient, and 3) the only source of heat is that being diffused from the well-mixed surface layer. Since our data consists only of inside corral measurements and weekly temperature profiles at the center of the lake, vertical advection and horizontal advection may be complicating

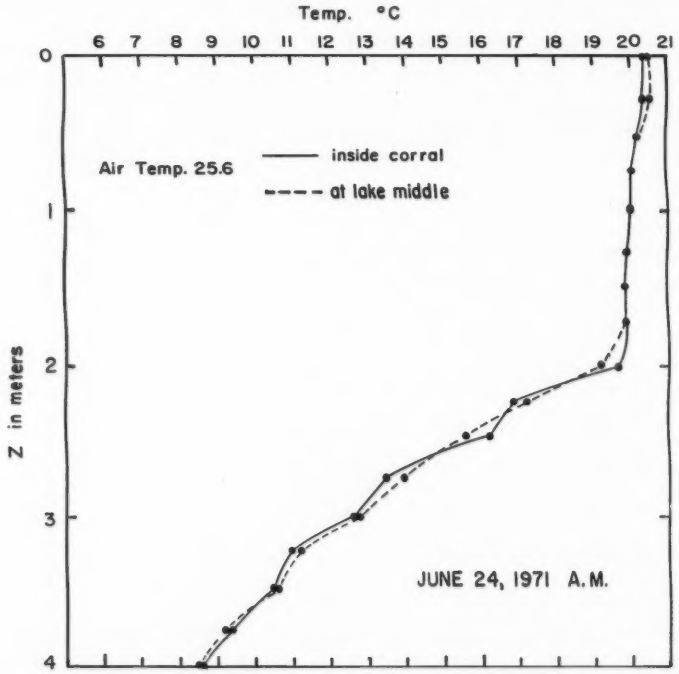

FIG. 9. Temperature vs. depth, inside the corral and in the middle of lake 227, taken on June 24, 1971.

factors. However, since lake 227 is small, the effects of wind-induced advective and entrainment processes and internal waves associated with prolonged wind shear are likely to be minimal. The temperature profile in Fig. 9, shows the homogeneous surface layer, both inside and outside the corral, with a definite temperature transition at the top of the thermocline. The shallow depth of the thermocline (2 m) reflects the unimportance of wind-induced mixing in the lake. The sharp temperature gradient (12 C in 2 m) greatly reduces vertical mixing through the thermocline of lake 227.

If we assume a one-dimensional model to be representative of lake 227, then a vertical eddy diffusion coefficient can be calculated at a certain depth interval from Fick's equation:

$$F(z, t) = -K(z, t)\,[dT/dz\ (z, t)]_t$$

where $F(z, t)$ = the total heat diffused through a unit horizontal layer per unit time and area at depth z and time t; dT/dz = the gradient of temperature vs. depth; K = the eddy diffusion coefficient. Five weekly dT/dz values are averaged over the time interval from June 29 to July 27, 1971; the individual dT/dz values used vary less than 20% from the average. This time interval corresponds to that of the radon and dye experiments. The calculated K values are 16.4×10^{-4} cm^2/sec, 15.1×10^{-4} cm^2/sec, and 14.3×10^{-4} cm^2/sec for the 3–4, 4–5, 5–6 m, layers, respectively. These results are similar to those obtained by Cowgill (1967) on Linsley Pond, Connecticut. The lake shows a similar temperature gradient in the thermocline as in lake 227, and yet has almost twice the area (9.4 ha) and a miximum depth of 14.8 m. This calculation reveals two important aspects of small lake mixing: 1) the K value calculated from a heat flux method is highly dependent on the heating period chosen; this observation was also made by Hutchinson (1941) on lake Mendota, where the weekly variations in K values calculated showed up to a 50% decrease from the K value determined from the whole heating season; 2) during the time interval used in this calculation, the effect of wind-induced turbulence must have been insignificant since the K values are approximately that of molecular heat conductivity which is 12×10^{-4} cm^2/sec. If the "real" eddy diffusion coefficient is less than 12×10^{-4} cm^2/sec, the heat method cannot be used, whereas the radon method, with a molecular diffusion coefficient equal to approximately 0.2×10^{-4} cm^2/sec, is still potentially valid. Therefore the difference in K values determined by the radon and dye method vs. the heat method could be explained by the fact that their molecular diffusion coefficients are different, giving the radon and dye methods validity at lower diffusion rates.

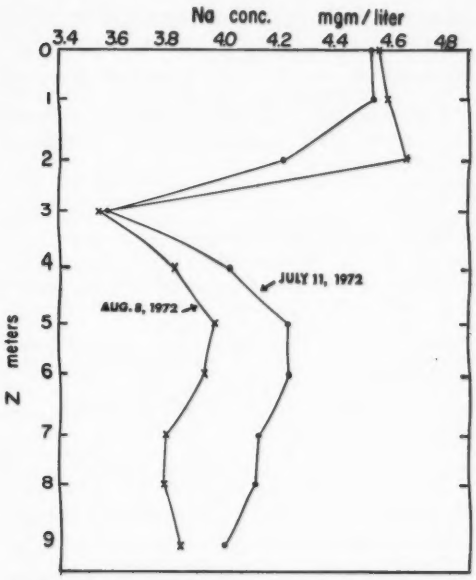

FIG. 10. Sodium concentration vs. depth in lake 227 on July 11 and August 8, 1972.

The "inhibiting" effects of the limnocorral on vertical mixing will depend on whether wind-

TABLE 4. Flux of Na to 2.5–3.5-m layer, July 11–August 8, 1972.

$\frac{dc}{dv}$ (2–3 m) (mgm/m^4)	$\frac{dc}{dv}$ (4–3 m) (mgm/m^4)	K (m^2/day)	Resultant Na conc (3m) (mgm/liter)	% of real Na conc
8.6×10^2	3.4×10^2	26×10^{-3}	0.44	1467%
8.6×10^2	3.4×10^2	10×10^{-3}	0.17	567%
8.6×10^2	3.4×10^2	4×10^{-3}	0.07	233%

induced turbulence, internal waves, and advection are more important than thermal stability. This evaluation will be different for every lake studied due to its particular morphometry, surroundings, etc. We feel that thermal stratification, which is similar inside and outside the corral, has the major influence on vertical mixing in lake 227 during the month interval of experimentation. This conclusion is supported not only by the above mentioned calculation, but also by a Na flux calculation from profiles taken on July 11, 1972 and August 8, 1972 (Fig. 10). The fluxes toward the minimum at 3 m, which was apparent in all four weekly profiles from July 11 to August 8, were calculated using the three K values determined by the temperature, dye, and radon methods. The minimum at 3 m is created by the weekly addition of $NaNO_3$ to the epilimnion of lake 227, as part of the eutrophication program (Schindler et al. 1973) and the possible addition to the volume below 3 m by particulate flux of undissolved $NaNO_3$ or release of Na from decay of sedimenting particles. The Na concentrations were determined by atomic absorption measurements with an accuracy of $\pm$ 1%. The results of the calculations using the gradient in Na from 2–3 m and 3–4 m as the best estimates of the fluxes at 2.5 m and 3.5 m are shown in Table 4. Only the change in concentration due to the fluxes at the rate predicted by the K determined by the radon method is in near agreement with the change observed over the 4-week period.

To conclude, we feel that the similarity of the thermal stratification both inside and outside the corral and the apparent lack of wind-induced turbulence are evidence that during the time interval of the corral experiments, the diffusion coefficients measured inside the corral are representative for vertical diffusion coefficients in the whole lake. Presently, this conclusion can be applied only to lake 227. When attempting a similar experiment on a different lake (i.e. a larger area) or during a different time (i.e. spring or fall), where the influence of wind is greater and the thermal stability of the thermocline is reduced, then the inhibiting effects of the corral walls must be considered. Further experiments are in progress.

The calculation that 8% of the total carbon loss from the epilimnion is returned using the K value determined by the radon method, is considered to be a much better estimate of the true value than the 50% estimate obtained using the temperature method. This conclusion applies only to highly stratified lakes which have very low rates of vertical mixing. It is suggested that the radon method is an excellent tool for monitoring the vertical mixing because of the simplicity of measurement and the sensitivity to very low rates of mixing. Changes in rates of mixing with time can also easily be detected due to the short, 6-day history recorded by the radon method.

Acknowledgments

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Diurnal Variation of Dissolved Inorganic Carbon and its Use in Estimating Primary Production and CO_2 Invasion in Lake 227

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SCHINDLER, D. W., AND E. J. FEE. 1973. Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in lake 227. J. Fish. Res. Board Can. 30: 1501–1510.

Standard in situ measurements of phytoplankton production and ^{14}C bottle bioassays gave erroneous results when applied to lake 227, a eutrophic softwater lake in the Canadian Shield. Errors were found to be due to diurnal variations in the degree of carbon limitation of phytoplankton, and to invasion of CO_2 from the atmosphere and hypolimnion.

A method based on diurnal measurements of dissolved inorganic carbon, community respiration, and invasion of CO_2, using gas chromatography, is described. Production by phytoplankton in lakes fertilized with nitrogen and phosphorus was found to be several times higher than in natural lakes of the area. Net production during summer stratification was found to equal invasion of CO_2 from the atmosphere.

The new technique should have application in other eutrophic low carbon lakes, where ^{14}C tracer techniques are encumbered by serious technical complications.

SCHINDLER, D. W., AND E. J. FEE. 1973. Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in lake 227. J. Fish. Res. Board Can. 30: 1501–1510.

Des mesures standards in situ de production de phytoplancton et des essais biologiques au ^{14}C à l'aide de bouteilles ont donné des résultats erronés lorsqu'appliqués au lac 227, lac eutrophe à eau douce du Bouclier canadien. Ces erreurs sont attribuables aux variations journalières de la limite imposée au phytoplancton par le carbone et à l'invasion du CO_2 de l'atmosphère et de l'hypolimnion.

Nous décrivons une méthode basée sur des mesures quotidiennes, par chromatographie en phase gazeuse, du carbone inorganique dissous, de la respiration par la communauté et de l'invasion du CO_2. La production de phytoplancton est plusieurs fois plus élevée dans les lacs fertilisés à l'azote et au phosphore que dans les lacs naturels de la région. La production nette durant la période de stratification estivale est égale à l'invasion du CO_2 atmosphérique.

Cette nouvelle méthode devrait pouvoir s'appliquer à d'autres lacs eutrophes à basses teneurs en carbone, là où l'emploi du ^{14}C est entravé par de sérieuses complications techniques.

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ONE of the major experiments of the ELA program (Johnson and Vallentyne 1971) is the controlled fertilization of lake 227 with nitrogen and phosphorus. In this hydrologically simple basin all important inputs and losses of the major nutrients are known; thus, the response of a whole system to a known nutrient loading can be determined. Schindler (1971) and Schindler et al. (1971, 1972a, 1973) reported the first results of this experiment. As expected, the phytoplankton standing crop increased dramatically after fertilization. Since the dissolved inorganic carbon (DIC) concentrations in the epilimnion of these lakes are the lowest ever reported in the literature, the increase in phytoplankton indicates that low concentrations of carbon will not prevent the eutrophication of a waterbody that receives adequate supplies of nitrogen and phosphorus.

However, in the course of the study a paradox emerged. Routine measurements of primary production by phytoplankton showed no increase over the unfertilized lakes in the area (Schindler unpublished data). The method of assaying primary production was the ^{14}C in situ incubation with a 4-hr midday exposure, as recommended by Vollenweider (1969) and described in detail by Schindler and Holmgren (1971). Since increases in standing crop are usually associated with increased primary production (Rodhe 1958), further investigation appeared to be in order.

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Moreover, in spite of the fact that carbon was shown not to limit the phytoplankton standing crop of lake 227, certain observations provided evidence of severe carbon limitation of photosynthesis. Schindler (1971) found that primary production of samples taken at midday was stimulated by additions of inorganic carbon. Further, the *p*H of the epilimnion increased markedly after fertilization and the partial pressure of gaseous CO_2 dropped several orders of magnitude. These are symptoms of a carbon deficit. Interpreted in the classical manner, these experiments and observations led to another paradox. Phytoplankton production in the lake appeared to be carbon limited, but the standing crops of phytoplankton had increased in response to nitrogen and phosphorus alone.

In an attempt to unravel the causal relations that led to these paradoxes, the series of experiments and observations described below were made in the summer of 1972.

Methods

The gas chromatographic technique (GC) used for dissolved inorganic carbon (DIC) analysis is described by Stainton (1973). The instrument was installed in a floating hut at the center of lake 227 for diurnal studies. Samples were drawn from the epilimnion of the lake (2 m in depth) using a constant-depth manifold with inlets spaced at ½-m intervals, and analyzed immediately. DIC samples from other lakes were taken with a peristaltic pump, put immediately into a cold box, and analyzed with another GC unit in the main laboratory, within an hour of collection.

The light incubator used was similar to that described by Fee (1973). It consisted of six 500-w tungsten—iodine vapor bulbs located at one end of a long table. A 5-cm thick Pyrex box filled with rapidly circulating cold water was placed directly in front of the lights to absorb heat. Experim.ntal bottles were placed on vertically rotating discs in four plexiglass chambers located at increasing distances from the lights. The amount and quality of light in each chamber was measured with an ISCO spectroradiometer so that photosynthetic response per unit light could be calculated. All samples were incubated at in situ temperatures. Carbon uptake was measured with the acidification and bubbling modification of the ^{14}C method (Schindler et al. 1972b).

Respiration rates were estimated by incubating water samples in 300-ml BOD dark bottles at ½-m intervals down to 2 m for 24 hr. The increase of DIC concentration over this time was assumed to be the daily respiration rate.

Two sets of incubator experiments were done. In the first, samples were taken at two depths several times during the day, injected with tracer amounts of $DI^{14}C$, and incubated at constant, identical light and temperature conditions. In the second, certain midday samples were enriched with DIC (as $NaHCO_3$), dissolved inorganic nitrogen (as $NaNO_3$), and dissolved inorganic phosphorus (as NaH_2PO_4), at concentrations of 1000, 50, 5 μg of the nutrient element per liter, respectively, then incubated and analyzed as before. The chemicals were added by injecting 100 μliters of concentrated nutrient solution for each element.

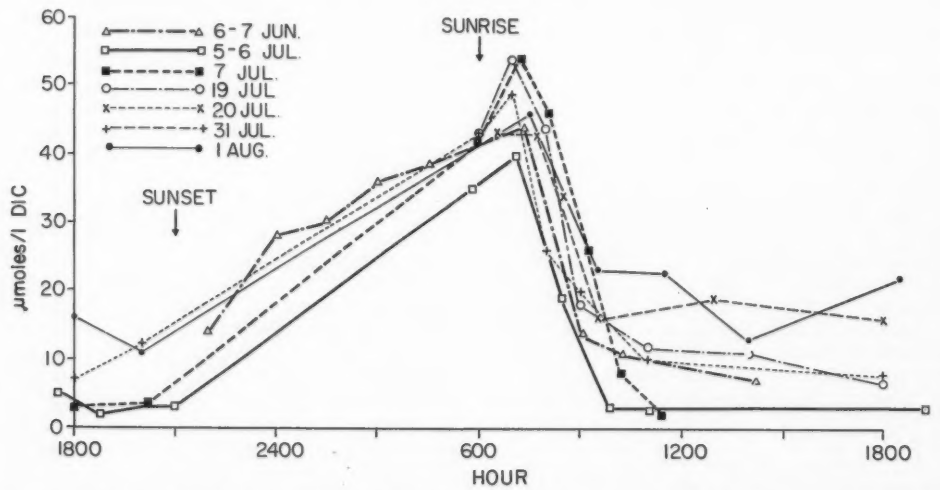

FIG. 1. Diurnal variations in concentration of DIC, lake 227, 0.5 m, summer of 1972. Note the regularity of the diurnal change, even though data span a 2-month period.

Results

Gas chromatographic studies revealed that epilimnetic DIC concentrations in midsummer increased continuously throughout the night, reaching maxima of 40–60 μmoles/liter by about an hour after sunrise. At this point, DIC began to decline rapidly (15–20 μmoles/liter/hr). By midmorning, DIC concentrations had reached 5–20 μmoles/liter, where they remained until sunset (Fig. 1). This general daily pattern was extremely regular, regardless of incident light.

Midday samples from depths that had low DIC showed extremely low carbon uptake by phytoplankton, while samples from early morning, with much higher DIC's, and from a depth where DIC was never depleted, were far more productive (Fig. 2). Furthermore, DIC-limited midday samples showed no response to variation in light intensity, while samples taken at other depths or times showed typical light responses (Fig. 2, Fee 1973).

Midday samples enriched with DIC exhibited a production curve identical to the early morning, while any sample not receiving DIC showed no

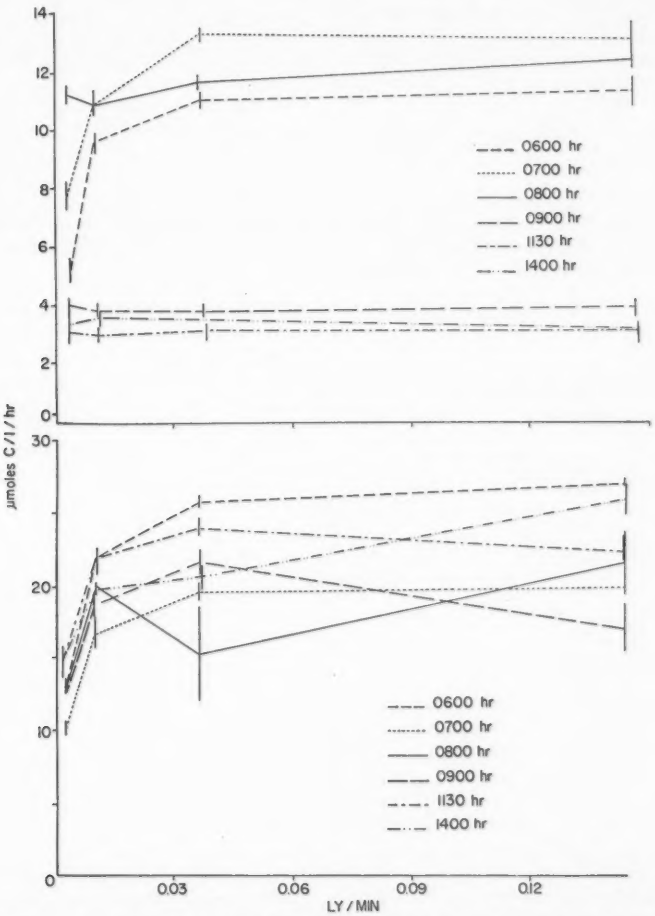

FIG. 2. Response of lake 227 phytoplankton taken at different times of day to four different light intensities in an incubator: top) 0.5 m (severe midday depletion of DIC); bottom) 2 m (little midday depletion of DIC). Pairs of points represent replicate bottles.

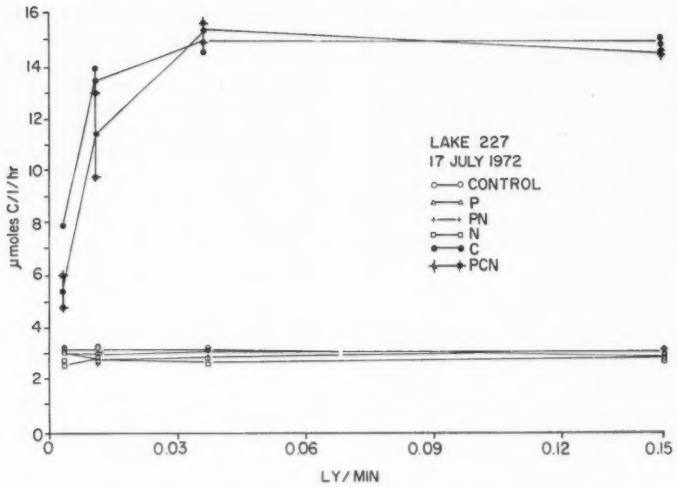

FIG. 3. Response by phytoplankton taken from lake 227 at midday to a variety of light and nutrient conditions. All samples were run in the light incubator at epilimnion temperature. Nutrient additions were 5 µg/liter PO_4–P, 50 µg/liter NO_3–N, and 1000 µg/liter HCO_3–C.

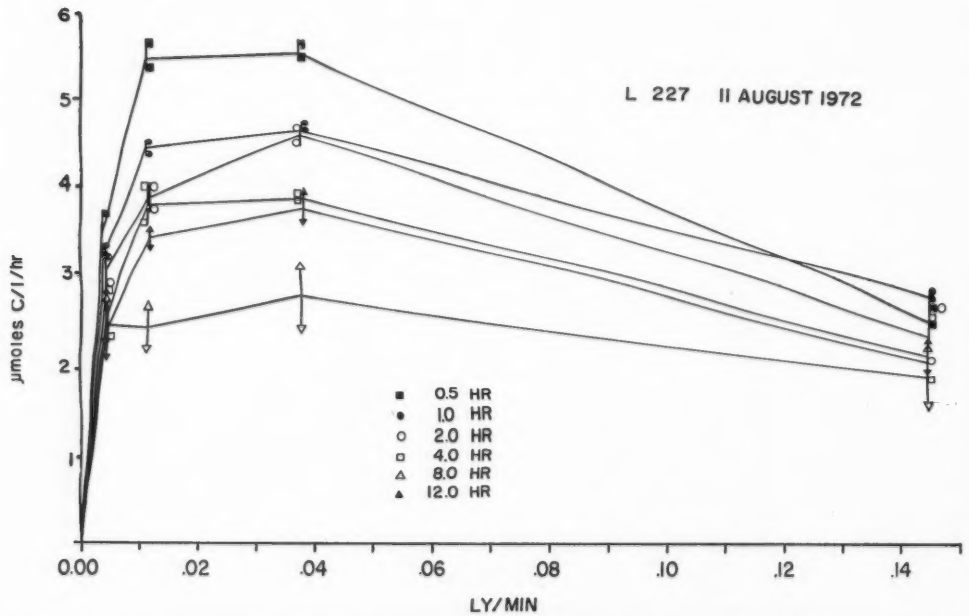

FIG. 4. Effect of incubation time on photosynthesis of samples from lake 227. The decline in average rate with time is due to depletion of DIC. Vertical bars connect duplicate analyses.

response, regardless of whether phosphorus and nitrogen were added (Fig. 3). One discrepancy apparent when comparing the diurnal curves with the incubator experiments was that the maximum rate of decline of in situ DIC was 15–20 μmoles/liter/hr, whereas the maximum rate of production observed in the incubator at optimum light was 12–15 μmoles/liter/hr, both with DIC enriched midday and with early morning samples from the epilimnion. An experiment performed later showed that the rate of production at the optimum light declined rapidly with increasing incubation time — perhaps as a result of carbon depletion in a bottle closed off from atmospheric invasion. The maximum rates of production, at optimum light and with half-hour incubation times, were about 30% higher than the routinely used 2–3 hr incubations at optimum light (Fig. 4). Correcting incubator results by this factor gave values close to those observed in situ.

Respiration rates varied from 2.4 to 122.4 μmoles/liter/day (Fig. 5).

Interpreting Diurnal DIC Measurements to Obtain Production Estimates

It was obvious from the above observations and from earlier measurements (Schindler et al. 1972a, 1973; Emerson et al. 1973) that invasion of CO_2 from the atmosphere was a major factor affecting the carbon dynamics of the lake.

Diurnal changes of DIC (Fig. 1) and estimates of respiration (Fig. 5) must therefore be corrected for invasion in order to estimate rates of primary production in lake 227. A simple model of the epilimnion DIC system (Fig. 6) shows the logic of our corrections. The DIC pool of the lakes' epilimnion is in dynamic equilibrium, supported by the rates of respiration and invasion from the atmosphere and hypolimnion, and depleted by the gross rate of primary production. Mathematically, the model is:

$$1) \qquad \frac{d[\mathrm{DIC}]_t}{dt} = \mathrm{R} + \mathrm{I} - \mathrm{P}_g$$

where $[\mathrm{DIC}]_t$ is the concentration of DIC at time t, R is the rate of respiration, I is the rate of invasion from the atmosphere and hypolimnion, and P_g is the gross rate of primary production. Rearranging and integrating gives an experssion for gross production in the lake over an arbitrary time interval:

$$2) \qquad \int_{t_1}^{t_2} \mathrm{P}_g \, dt = (t_2 - t_1)\,(\mathrm{R} + \mathrm{I}) - [\mathrm{DIC}]_{t_2} - [\mathrm{DIC}]_{t_1}).$$

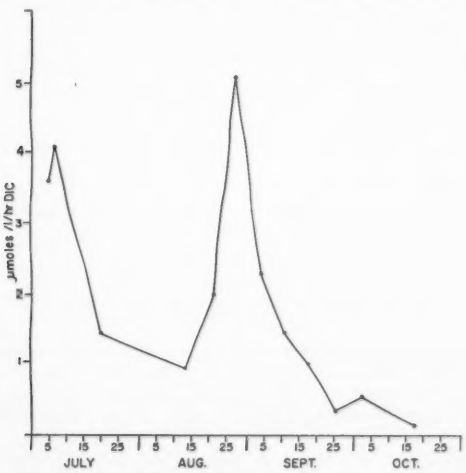

FIG. 5. Respiration in dark bottles during the period of study for lake 227. Results are averages for the upper 2 m of the lake.

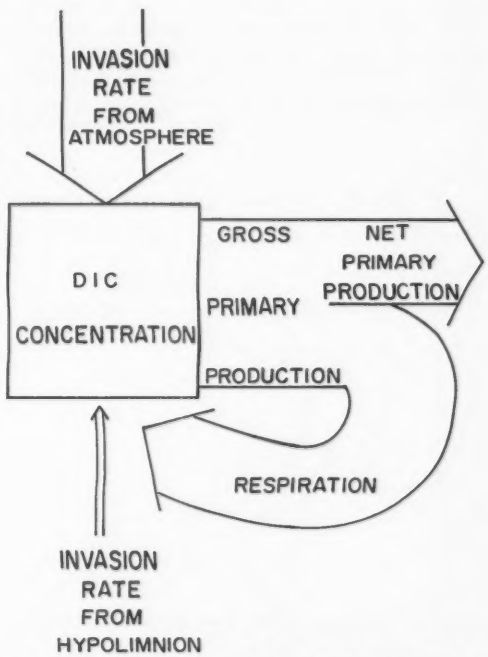

FIG. 6. The DIC model of lake 227. Respiration (R) and Invasion (I) increase the DIC concentration and gross production decreases it. If the DIC concentration is the same at the beginning and end of a given time interval, then gross production over that interval is R + I. If there is a net change in the DIC concentration, then this change must be subtracted from (R + I) to obtain gross production.

Net production is this expression with the respiration term omitted.

It follows from the model that the maximum rate of production occurred in the early morning, since this was the time of greatest change of concentration of DIC. This rate is a measure of the photosynthetic potential of the system in the absence of any DIC limitation. For most practical calculations, the daily rates of gross and net production are of greater value. Since there is little or no net change of the DIC concentration over a 24-hr period in summer (Fig. 1) the last term in equation (2) is zero and daily gross production is equal to the daily rate of respiration plus invasion while net production is the daily rate of invasion.

To obtain production estimates it is thus necessary to measure the rates of both respiration and invasion of DIC. We used the following procedures and assumptions for estimating these rates:

In spite of the approximately 10× diurnal change in concentration of DIC, a maximum change of only 0.1–0.2 *p*H units was observed over the diurnal cycle. Since *p*H was consistently near 10, invading CO_2 was quickly hydrated to HCO_3^- and $CO_3^=$ and the partial pressure of CO_2 should not show substantial diurnal changes. We therefore assumed that the rate of atmospheric invasion of CO_2 was similar both night and day. Likewise, turbulent transport of DIC through the thermocline from the hypolimnion was assumed to be invariant over the diurnal cycle. We further assumed that there was no difference in algal or bacterial respiration rates in light and dark. Zooplankton were probably unimportant contributors to community respiration since populations were very low.

The in situ rate of DIC increase during the night (Fig. 1) was assumed to be solely due to the sum of the rates of invasion and respiration (R + I). Subtracting the respiration rates (R) obtained in dark bottles allowed I to be estimated.

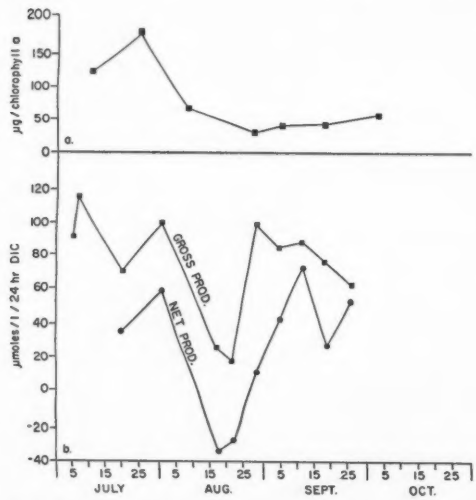

FIG. 7. Average phytoplankton standing crop and production for the upper 2 m of lake 227: a) phytoplankton standing crop as µg chlorophyll/liter; b) gross and net production during the study period. For method of calculation, see the text. Evasion of DIC at the air–water interface is responsible for the negative net production results in mid-August.

PRODUCTION ESTIMATES

Gross production (= Invasion + Respiration) ranged from 20 to 120 µmoles of DIC/liter/day (Fig. 7). The maximum rate of gross production over the day occurred just after sunrise and averaged 20–25 µmoles DIC/liter/hr during summer. This rate is far higher than all but the most eutrophic waterbodies. Net production varied from –36 to 72 µmoles DIC/liter/day. These rates are expressed in units of µmoles/liter of epilimnion; to convert them to areal units for comparison with other data multiply by 24 to get mg C/m².

On two dates in mid-August respiration exceeded gross production, so that calculated net production was negative. This occurred just after the algal bloom decreased from 176 to 28 µg of chlorophyll

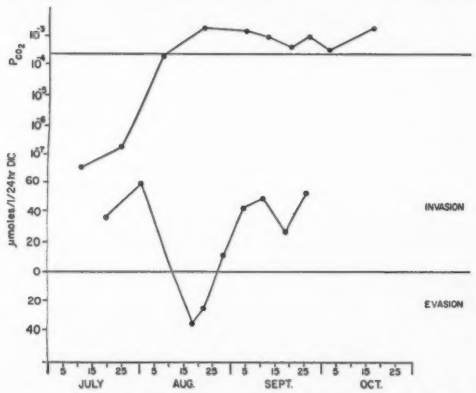

FIG. 8. Partial pressure (pCO_2), invasion and evasion of DIC from atmosphere and hypolimnion to the epilimnion of lake 227. Invasion and evasion values are averages for the epilimnion (upper 2 m), calculated from diurnal curves and dark bottle results as explained in the text. The period of high evasion in late August corresponds with the decline of standing crop, high respiration, and low production. Straight horizontal lines represent atmospheric equilibrium.

a/liter due to a period of cool and cloudy weather (Fig. 7). Protozoans and bacteria were abundant at the time. The DIC concentration of the epilimnion increased from 20 to 30 μmoles/liter immediately before the crash to 250–300 μmoles/liter, causing the epilimnion partial pressure of CO_2 (p_{CO_2}) to rise above the p_{CO_2} of the atmosphere (Fig. 8). More CO_2 left the lake by evasion than was fixed by phytoplankton at this time. The increase of the epilimnetic DIC concentration in mid-August could not be accounted for by deepening of the thermocline (Fig. 9) and therefore a cessation of production concomitant with an increase in respiration may be logically postulated.

Sources of DIC for Photosynthesis

It is of interest to determine whether invading DIC originated from the atmosphere or the hypolimnion. Using radon gas, Hesslein and Quay (1973) measured a coefficient of eddy diffusion for the thermocline region in lake 227 of 2–10 × 10^{-4} cm^2/sec (= 17–86 × 10^{-4} m^2/day) during July and August of 1972. Using the maximum observed gradient of DIC of 500 μmoles/m (Fig. 9b), Fick's law predicts flux rates of 0.8–4.3 mmoles/m^2/day. To an epilimnion 2 m in depth, this is 0.4–2.2 μmoles/liter/day. Because gross production was 20–120 μmoles/liter/day, hypolimnetic influx was therefore unimportant as a source of DIC for phytoplankton photosynthesis during summer stagnation. When stratification began to break down in autumn, however, hypolimnetic DIC was undoubtedly an important source of supply (Fig. 9b).

Atmospheric invasion, with rates of 24–60 mmoles/m^2/day (12–30 μmoles/liter/day in an epilimnion 2 m deep), was far more important as a DIC source during summer. These rates of atmospheric invasion agree well with the observed increase in carbon in the lake (46 mmoles/m^2/day) (Schindler et al. 1973) and calculations based on the exchange of radon gas (17 ± 8 mmoles/m^2/day) (Schindler et al. 1972a, Emerson et al. 1973).

Community respiration was also an important source of DIC for phytoplankton, furnishing 2.4–122.4 μmoles/liter/day.

Carbon did not appear to limit production in lake 227 after the DIC pool increased in late August (Fig. 10). There was a continuous decline of DIC concentrations throughout the day at this time instead of the sharp drop of DIC concentrations in early morning which was observed consistently in summer.

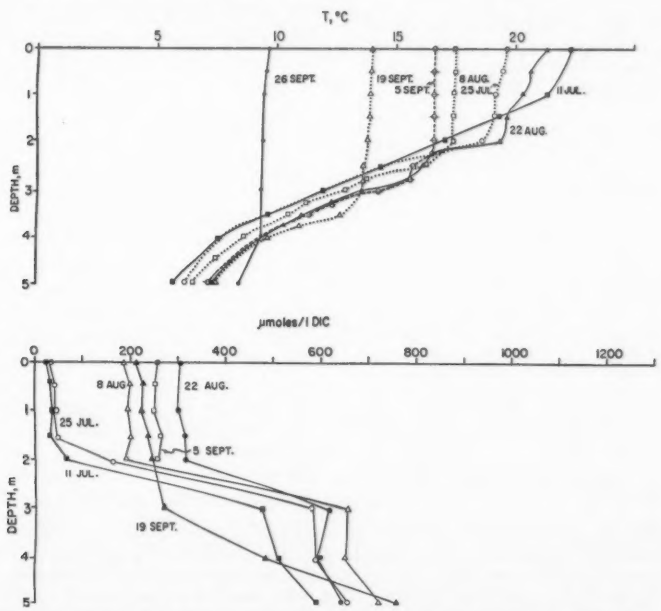

FIG. 9. Erosion of the thermocline in lake 227 during the period of study: a) as shown by temperature profiles; b) as shown by DIC profiles.

Diurnal DIC curves similar to Fig. 1 appear to be a certain indication that carbon is the nutrient immediately limiting to phytoplankton production. To determine the extent of the phenomenon in other ELA lakes, DIC concentrations were measured in early morning (0630 hr) and late afternoon (1730 hr) on August 1, 1972. If depletion of DIC due to photosynthesis occurred, then concentrations at these times should have been significantly different. The following table shows the results (units are μmoles of DIC/liter and are euphotic zone averages):

	time		
lake	0630	1730	net change
114	50	43	7
227*	46	22	24
239	116	115	1
240	95	88	7
304*	41	14	27
305	132	129	3

*Lakes being artificially fertilized with N and P.

Morphometry for the above lakes is given by Brunskill and Schindler 1971. Only lakes 227 and 304 showed the expected large change in DIC concentrations. These are lakes that were being heavily fertilized with nitrogen and phosphorus. Unmodified lakes did not show symptoms of carbon limitation, and had much lower phytoplankton production.

Discussion

The explanation of the paradoxes is apparent. There had been no increase in measured primary production in lake 227 following fertilization because the in situ incubations were always performed between the hours of 10:00 and 14:00. As shown by the incubator results (Fig. 2a and 3), there was inadequate DIC to support photosynthesis during this period. Samples taken in the early morning or samples enriched with DIC had very high rates of production. Primary production had certainly increased as a result of fertilization with nitrogen and phosphorus. Blind adherence to a standard methodology had, however, obscured the fact. Special methods, such as the diurnal technique

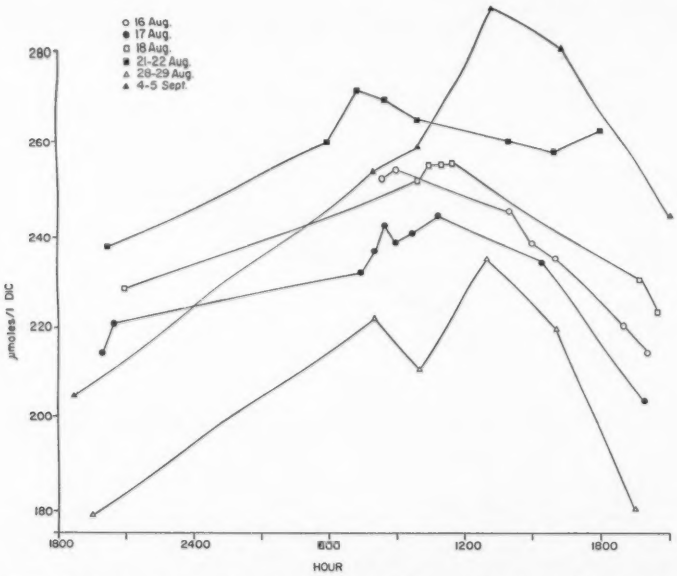

FIG. 10. Diurnal curves for lake 227 after DIC concentration had increased due to the decline in phytoplankton in late August and the erosion of the thermocline in early September. Note that the midday low in production observed during summer stratification (Fig. 1) has disappeared, and that depletion continues until late afternoon. The extreme regularity of the daily cycle found in midsummer has also disappeared, and differences in curves appear to reflect changes in the light regime.

used here will be necessary in lakes with low DIC concentrations and relatively high production.

While ^{14}C nutrient bioassays done at midday demonstrated that DIC was immediately limiting to primary production, they did not give an accurate assessment of the nutrient requirements of lake 227 phytoplankton, since high production during the early morning hours due to overnight regeneration of DIC by respiration and invasion was sufficient to maintain a large standing crop. Obviously, great care must be taken when drawing conclusions about daily, monthly, or seasonal phytoplankton dynamics from in situ measurements or bioassays of a few hours duration. In such situations basic assumptions of the ^{14}C tracer method may be violated. For example, 30–50% of the added ^{14}C (1 μc was added to a 125-ml sample) was incorporated into photosynthetic products in some of the incubator experiments. Obviously, in such cases the ^{14}C added is no longer a tracer since the ^{14}C available for uptake significantly declines throughout the course of the experiment. Further, if the DIC pool is rapidly turning over (as shown by Schindler et al. 1973), the amount of ^{12}C available for uptake, which is required for calculating photosynthetic rates from ^{14}C uptake, is not simply the DIC concentration (as assumed by the standard formulae). In such situations, the ^{12}C available for uptake is the DIC concentration multiplied by the rate constant of the DIC pool (i.e. the proportion of the pool regenerated in a unit time) multiplied by the incubation time. It is not a simple matter to estimate the rate constant of the DIC pool, and errors resulting from ignoring this may be quite significant. Clearly, an altogether different approach is required for estimating primary production and carbon dynamics in such waters. The diurnal curve method described here offers such a method.

It is unlikely that an additional supply of carbon would have allowed an increase in phytoplankton standing crop. The maximum standing crop which can be maintained in the euphotic zone of a natural lake is usually thought to be about 300 μg/liter, as chlorophyll *a*. Lake 227 reached or exceeded this figure in July and mid-August of 1972 without any carbon enrichment. Results of enrichment experiments conducted in meter-diameter tubes in 1969 and 1970 (Schindler et al. 1971 and unpublished data) confirmed this observation. Carbon additions caused little or no response beyond that attainable by fertilization with phosphorus and nitrogen alone.

The simplistic approach often taken to nutrient limitation problems is inappropriate for accurate analysis of many eutrophication-related problems. As O'Brien (1972) has pointed out, one nutrient may control the rate at which standing crops increase, while another governs the maximum standing crop which can be supported. Only the latter nutrient is of any practical significance to the control of eutrophication. The relative importance of nutrients may also change seasonally or even diurnally. Phosphorus and carbon in lake 227 provide a good example of these phenomena.

Our work also demonstrates that perhaps the most relevant question to eutrophication, i.e. "what nutrient has caused standing crops to increase?," cannot be easily answered after a lake has been altered. Perhaps the only way nutrient bioassay experiments can be useful as a management tool is to conduct such experiments before a water body is disturbed. Only by knowing the nutrient requirements of the undisturbed ecosystem can the manager hope to predict what nutrient control scheme is most likely to be effective in maintaining water quality.

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Production of Epilithiphyton in Two Lakes of the Experimental Lakes Area, Northwestern Ontario

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Two new techniques for measuring photosynthesis by benthic algal flora in waters low in dissolved inorganic carbon are described. The first uses gas chromatography to measure changes in DIC in incubation chambers directly. The second is a variation of the usual ^{14}C procedure, in which disappearance of ^{14}C from the water is measured by liquid scintillation instead of uptake of ^{14}C by the algae. This procedure is simpler than measuring the uptake of ^{14}C, because digestion and/or combustion of samples is not necessary. Results are compared with the commonly employed ^{14}C uptake and O_2 release techniques.

Tests showed that heterogeneity of substrate was the major source of variation in in situ results, being large enough to make interpretation of seasonal effects and other causal factors extremely difficult.

Annual production by epilithiphyton in two natural lakes in the Experimental Lakes Area (ELA) was 5.19 g C and 5.18 g C/m^2 of substrate annually for lakes 239 and 240, respectively. These are the lowest values recorded for freshwater lakes at temperate latitudes.

Because DIC and O_2 concentrations could be measured simultaneously, it was possible to calculate photosynthetic quotients on several dates. These were extremely high, averaging 2.6 for the summer of 1971.

SCHINDLER, D. W., V. E. FROST, AND R. V. SCHMIDT. 1973. Production of epilithiphyton in two lakes of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 30: 1511–1524.

Nous décrivons deux nouvelles méthodes de mesure de la photosynthèse à l'aide de la flore algale benthique dans des eaux à faible teneur en carbone inorganique dissous. La première fait appel à la chromatographie en phase gazeuse pour mesurer les changements de CID directement dans les chambres d'incubation. La seconde est une variante de la technique usuelle au ^{14}C, dans laquelle la disparition du ^{14}C de l'eau est mesurée par scintillation liquide plutôt que par captation du ^{14}C par les algues. Cette procédure est plus simple que la mesure de la captation du ^{14}C, puisqu'elle ne requiert pas de digestion et/ou de combustion des échantillons. Nous comparons nos résultats avec ceux obtenus avec les méthodes communément utilisées de captation du ^{14}C et de libération d'O_2.

Des essais démontrent que l'hétérogénéité du substrat est la principale source de variation des résultats in situ, au point de rendre extrêmement difficile l'interprétation des effets saisonniers et autres facteurs.

La production annuelle de l'épilithiphyton dans deux lacs naturels de la région des lacs expérimentaux (ELA) est de 5.19 g C et 5.18 g C/m^2 de substrat annuellement pour les lacs 239 et 240 respectivement. Ce sont les plus basses valeurs jamais enrégistrées dans des lacs d'eau douce des latitudes tempérées.

Puisque les teneurs en CID et en O_2 peuvent être mesurées simultanément, nous avons pu calculer les coefficients photosynthétiques à plusieurs dates. Les valeurs obtenues ont été extrêmement élevées, soit 2.6 en moyenne pendant l'été 1971.

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THE few published studies of production by natural communities of periphyton[2] have used either the oxygen or the ^{14}C technique (reviewed by Wetzel 1965; see also Hargrave 1969, Allen 1971, Hickman 1971, Gruendling 1971). The two techniques have not been compared for aufwuchs communities, although agreement between them for macrophytes was found to be poor (Wetzel 1965) and for phytoplankton variable (reviewed by Romanenko 1967).

For phytoplankton, the ^{14}C method is generally thought to measure something between net and

[2]The terminology recommended in reviews by Wetzel 1964 and Hickman 1971 is used in this paper.

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gross production, depending on light intensity and duration of the incubation period (Vollenweider 1969). Wetzel (1965) presented some evidence that ^{14}C uptake equals net production for aquatic macrophytes. Tests on periphyton are totally lacking. Furthermore, agreement between the ^{14}C and oxygen methods can be validly tested only if the photosynthetic quotient

$$\left[PQ = \frac{O_2 \text{ evolved, moles}}{CO_2 \text{ uptake, moles}}\right]$$

is known, bacause knowledge of PQ is required if both oxygen and carbon data are to be expressed in comparable units.

Periphyton communities are notoriously difficult to manipulate for realistic production measurements. Investigators have had to use either small enclosed natural areas, or glass slides, or other artificial substrates. Although no one has compared rock with artificial substrates, Tippet (1970) found little similarity between the periphyton communities on macrophytes and on glass slides, either in species composition or numbers per unit area. He concluded that results from studies of artificial substrates cannot be validly extrapolated to natural communities. In a few cases where filamentous algae have been studied, the algae have been clipped from their substrate and incubated in bottles similar to those used for phytoplankton (for example, Bodin and Nauwerck 1969). Heterogeneity or artificiality of substrate therefore has made interpretation of these studies difficult.[3] Wetzel (1965) reviews the subject.

The purpose of this study was to measure the importance of epilithiphyton production in the overall production of two Canadian Shield lakes and to improve the technology for making such measurements in waters low in dissolved inorganic carbon. Techniques for simultaneous measurements with direct O_2 and CO_2 methods were developed to allow calculation of PQ (photosynthetic quotients) and to allow direct calibration of the ^{14}C method. The nature of the bottom substrate in these studies made it possible to work with fairly large chambers, so that the effect of substrate heterogeneity should be considerably less than in previous studies.

Description of the Study Area

Periphyton in lakes of the Experimental Lakes Area are abundant in rocky littoral areas. Seasonal succession and chemical composition of algae in such areas of four ELA lakes were studied by Stockner and Armstrong (1971), who found that diatoms were the predominant algal group. Two of these lakes, lake 239 and lake 240 (= Rawson and Hayes lakes, respectively), were chosen for experiments reported here. Both have large littoral areas of stones ranging in diameter from approximately 5–50 cm, which are covered with an abundance of epilithiphyton. The stones are chiefly granitic, similar to the bedrock of the drainage basins of the lakes.

Both lakes are small (area = 56.1 and 44.1 ha, mean depth = 10.5 and 6.1 m, for lakes 239 and 240, respectively). Other morphometric and geological characteristics may be found in Brunskill and Schindler (1971).

Phytoplankton production in both lakes was negligible below a depth of 6 m due to attenuation of light by dissolved humic matter (Schindler and Holmgren 1971, Schindler 1972 and unpublished data), and rocks below that depth were usually covered with flocculent forest debris. Measurements of epilithiphyton production were therefore confined to depths of 0.1, 1, 2, 3, 4, and 5 m in both lakes. The area of the rocky zone at depth 0–6 m in Lake 239 is 8.78 ha, or 15.6% of the surface area of the lake (Fig. 1). The corresponding value for lake 240 is 7.63 ha, or 17.3% of surface area (Fig. 2).

Measurements in 1971 were begun in June and continued until November, when a rim of ice had formed on both lakes. Work on lake 239 was continued from ice-out (late April) until mid-June in 1972 to give a full season's data. Measurements were usually made weekly at each of three stations in lake 239 (Fig. 1) and at one station in lake 240 (Fig. 2).

Methods

Enclosure of the Communities

Samples were incubated in plexiglass containers 0.5 × 0.2 m in area and 0.1 m deep (Fig. 3). Each chamber was positioned by a SCUBA diver at the desired depth as marked by a permanent buoy. The door and end-ports were left open. The bottom of the chamber was then covered with rocks from the marked area, after which the door and the end-ports were sealed. If rocks were carefully moved, the community in the chamber closely resembled that outside, and it is assumed throughout that production per unit area inside the chamber was similar to that of the surrounding area. Two light chambers and one dark chamber were normally used at each depth. Chambers were darkened by enclosing them in aluminum foil and several layers of opaque polyethylene sheeting. Exposure was for 4 hr unless otherwise stated, usually between 1000 and 1500.

[3]No one appears to have compared the primary production of climax periphyton communities which develop on natural versus artificial substrates.

Methods of Production Measurement

Four methods were applied simultaneously to the above chambers; direct measurement of changes in dissolved inorganic carbon (DIC = CO_2 + HCO_3 + CO_3 + H_2CO_3) and O_2, rate of ^{14}C disappearance from water, and rate of ^{14}C uptake by epilithiphyton.

The DIC method was used as the "standard" against which other methods were compared, since most authors have used carbon units to express their production data, and all other methods include some untestable assumptions to convert to carbon units. We are not aware of any other field study of periphyton production where DIC changes have been measured directly.

Incident light at the surface was measured with an Eppley pyrheliometer mounted at the center of lake 239. Light at depth was calculated from incident light and the vertical extinction coefficient of light in water, as obtained with a Whitney submersible photometer (cadmium sulfide cell).

Measuring changes in oxygen alone has several shortcomings. Energy transfer to consumer trophic levels is not usually expressed in oxygen units, and assumptions about photosynthetic and respiratory quotients (PQ

Fig. 1. Epilithiphyton-covered rocky areas in the littoral zone of lake 239. The map is extended from Stockner and Armstrong (1971). Shading indicates areas extrapolated from north, west, and east benthic stations in order to calculate productivity of the entire rocky zone, and arrows indicate sites of production stations.

and RQ) must be made to convert data to units of carbon or energy. In addition, working with samples supersaturated with oxygen presents analytical problems. Moreover, oxygen bubbles produced by photosynthesizing plants often cling to the plants and do not rapidly dissolve in waters, causing production to be underestimated (Wetzel 1965).

Carbon-14 measurements are difficult to interpret, because there is still no conclusive evidence about whether the technique measures net or gross production or something in between. In addition, there are problems with self-absorption if plant materials must be radioassayed for ^{14}C uptake. Techniques for overcoming self-absorption are tedious and expensive. Radioassay of periphyton after incubation is referred to below as the "^{14}C uptake" method. In addition, we were able to monitor the disappearance of dissolved inorganic ^{14}C from the water, as outlined later. This technique will be referred to as the "^{14}C disappearance" method.

Sampling and Analysis of Dissolved Oxygen and Inorganic Carbon

After a chamber was filled with rocks, each stirring handle (Fig. 3) was rotated 100 times to ensure thorough mixing. Duplicate samples for CO_2 and/or O_2 analyses were then taken by a SCUBA diver with 50-ml disposable polyethylene syringes, drawing each sample through the serum bottle stopper in the top of the chamber. Water to replace that withdrawn leaked into the chamber around the door gasket. The amount was insufficient to cause significant changes in concentration of O_2 or CO_2 in the chamber. Syringe samples for oxygen analysis by the azide modification of the Winkler method (Anon. 1965) were fixed under water, by injecting 0.5 ml each of $MnSO_4$ and alkaline iodide into the syringe-needle aperture with smaller syringes. Once the syringes were returned to the laboratory, sulfuric acid was added in the same way, and after shaking, 50 ml were measured into a volumetric flask and titrated with N/20 sodium thiosulfate and a micrometer burette.

Samples for analysis of DIC were returned immediately to the central field laboratory. Samples were acidified to *p*H 3.5, equilibrated with helium in the sampling syringe, and injected into a Fisher model 29 gas chromatograph equipped with a 5-ml sample loop and a 6 ft × ¼ inch (1.8 m × 63.5 mm) column of Porapak Q. The method is described in detail by Stainton (1973).

Similar syringe samples for CO_2 and O_2 analysis were taken after incubation (usually 4 hr; again after 100 rotations of each stirring handle). The volume of water in each incubation chamber was determined by bringing chambers still filled with rocks to the surface after incubation, and pouring the entire volume of water of

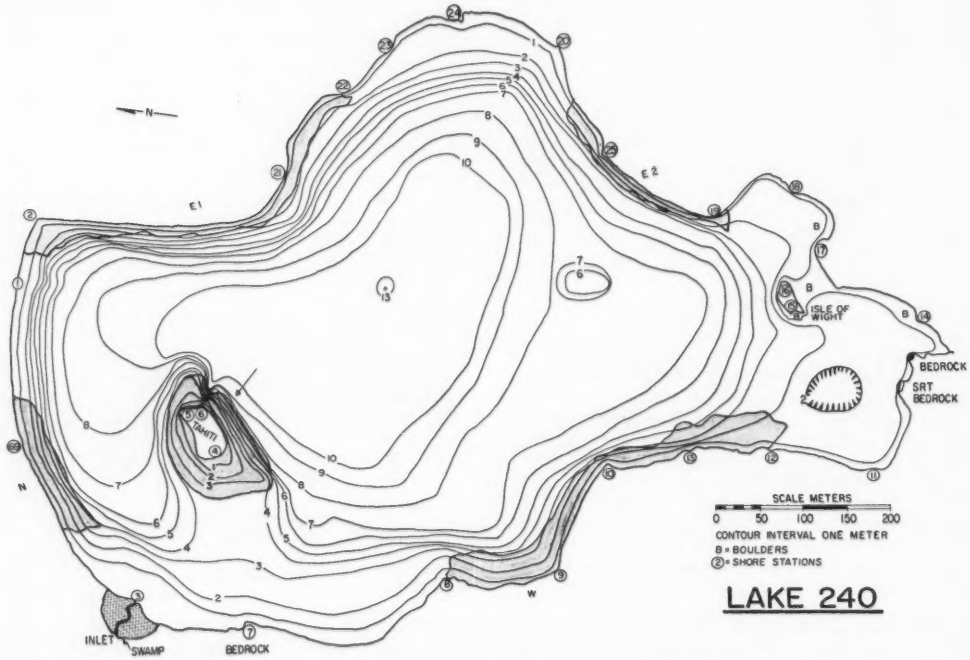

FIG. 2. Epilithiphyton-covered rocky areas in the littoral zone of lake 240. The map is extended and corrected from Stockner and Armstrong (1971).

FIG. 3. Plexiglass chambers used for enclosing rocks for production studies. Chambers were darkened for respiration and dark-uptake experiments by covering them with a layer of aluminum foil and opaque polyethylene. In order to measure the volume of water after incubation, one of the rubber bungs is pulled from the chamber, allowing water to flow into a 10-liter plexiglass cylinder as shown. Since the volume of the chamber is exactly 10 liters, subtracting the water volume allows the volume of rock to be determined. In our experiments, chambers usually contained 2.5–3.5 liters of rock and 6.5–7.5 liters of water.

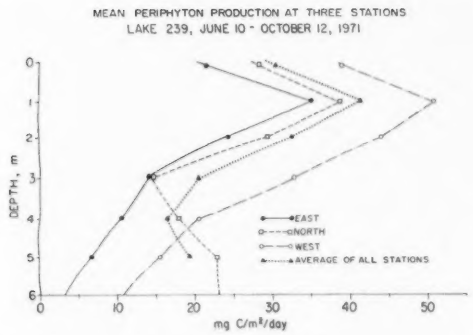

FIG. 4. Average net production by epilithiphyton at three stations, lake 239, June 10–October 12, 1971.

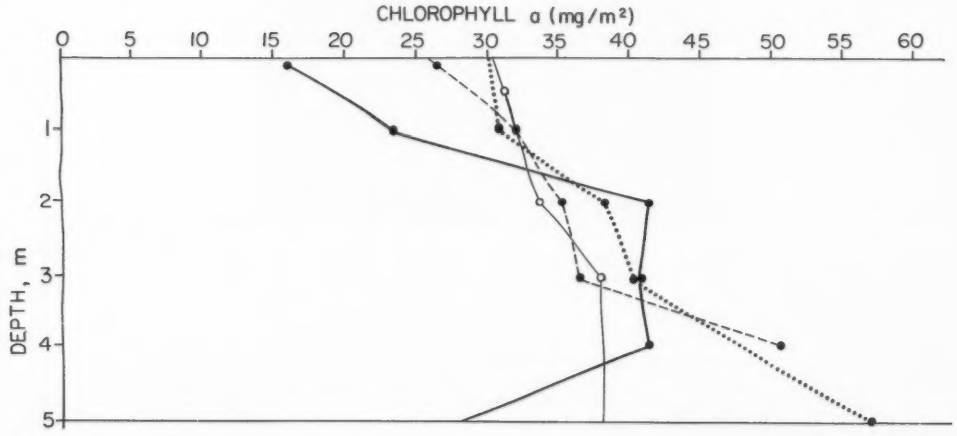

FIG. 5. Average standing crop of epilithiphyton as chlorophyll-*a*, lakes 239 and 240, June 10–October 12, 1971. Solid line — solid dots = L239 West Station; dashed line — solid dots = L239 East Station; dotted line — solid dots = L239 North Station; light solid line — open dots = L240 South side of island.

each chamber into a 10-liter graduate cylinder constructed from a piece of plexiglass tubing (Fig. 3). Since the volume of each chamber was exactly 10 liters, the volume of rocks enclosed in each chamber could also be calculated.

Chlorophyll Measurements

After exposure, rocks from one chamber at each incubation depth on each date were scrubbed into 4 liters of lake water, using a wire brush. Two 10-ml aliquots of well-mixed scrubbings were then filtered onto Whatman GF/C glass-fiber filters, for fluorometric determination of chlorophyll-*a* as described by Schindler et al. (1971). Results were calculated as milligrams chlorophyll-*a*/m² of bottom surface, as outlined below.[4]

Production by the ^{14}C Method

After chambers were filled with rocks, 2 ml (20 μC) of ^{14}C as Na_2CO_3 were injected into each chamber through the serum bottle stopper, using a small syringe. Each stirring handle of the chamber was rotated 100 times, then two 2-ml subsamples were drawn from each chamber with a small disposable plastic syringe, and placed in scintillator vials containing dioxane-base fluor (Schindler and Holmgren 1971). A similar stirring and sampling was done at the end of a 4-hr exposure. Chambers were stirred occasionally throughout the experiment. At the end of the incubation, rocks were scrubbed into 4 liters of lake water using a wire brush, and aliquots of the periphyton scrubbings were taken for digestion in NCS (Amersham-Searle Corporation) and liquid scintillation counting. Periphyton production could therefore be calculated either from the rate of ^{14}C disappearance from the water, or the rate of ^{14}C uptake by periphyton. Experiments showed that the amount of ^{14}C recovered from the water and from rock scrubbings nearly equalled that injected, and that residual radioactivity of rocks was low, so adsorption of ^{14}C to the chamber walls was negligible. Calculations are set out below.

Statistical Tests

No previous authors have published the precision of their periphyton production techniques, or tried to analyze the variation in results. Since this is necessary for any critical evaluation of causal factors, we performed the following tests.

On one occasion 40 syringes were filled with water from the same depth and analyzed for DIC in the usual manner. An average of 142.1 μmoles/liter was obtained, with a standard deviation of 3.05 μmoles/liter, or a coefficient of variation of 2.1%.

On another occasion (August 5), five replicate chambers were filled at a depth of 1 m, and a second five at 2 m. All were incubated for 4 hr, and production was determined by all four techniques. Results should give some index of variation due to substrate variability at a given depth. Standard deviation averaged 25.3% of the mean, with greatest variation occurring with the O_2 method (average coefficient of variation = 34.8%) and least with the ^{14}C uptake method (average coefficient of variation = 18.5%). While this variation is rather large, we judged it acceptable for these preliminary experiments.

Calculation of Results

Direct CO_2 and O_2 methods — Assuming areal production to be proportional to surface light (see below), daily net production (Pr_n) is:

$$(1) \qquad Pr_n = \left[\frac{\Delta C_g \, V}{A}\right]\left[\frac{I_o}{I_e}\right]$$

where ΔC_g is the absolute change in concentration of DIC or O_2 in mg/liter or μmoles/liter during an experiment, V is volume of water in the chamber in liters, A is the area of the chamber bottom in m², I_o is the daily incident solar radiation, and I_e is the incident solar radiation during exposure as measured with an Eppley pyrheliometer. Pr_n is expressed in two ways, as mg C/m²/hr, for comparison with other production results, and in μmoles/liter, for calculation of photosynthetic and respiratory quotients. Daily respiration is:

$$(2) \qquad R = \left[\frac{\Delta C_g \, V}{A}\right]\left[\frac{24}{e}\right]$$

where e is the exposure time in hours and ΔC_g is the absolute change in O_2 or CO_2 observed in the dark chamber.

"^{14}C disappearance" method — Net production is:

$$(3) \qquad Pr_n = \left[\frac{\Delta^{14}C \;\; C_{t=o}}{^{14}C_{t=o}}\right]\left[\frac{V}{A}\right]\left[\frac{I_o}{I_e}\right]$$

where $^{14}C_{t=o}$ is the ^{14}C activity (dpm/ml) of chamber water at the beginning of an experiment, $\Delta^{14}C$ is the difference in radiocarbon content of the water between beginning and end of the experiment (dpm/ml), and $C_{t=o}$ is the concentration of DIC at the beginning of an experiment. All other symbols were defined above.

"^{14}C uptake" method — Net production is:

$$(4) \qquad Pr_n = \left[\frac{V}{A}\right]\left[\frac{^{14}C_p \;\; C_{t=o}}{^{14}C_{t=o}}\right]\left[\frac{I_o}{I_e}\right]$$

where $^{14}C_p$ is the total ^{14}C content of the periphyton in the chamber after exposure (dpm), $^{14}C_{t=o}$ is the total ^{14}C activity (dpm) in the chamber at the beginning of an exposure and other symbols are as used above.

[4]Chlorophyll-*a* values given here are uncorrected for phaeophytins and other degradation products. Realistic corrections for phaeo-pigments are nearly impossible to obtain on a routine basis (Daley et al. 1973, ELA staff unpublished).

Calculation of photosynthetic and respiratory quotients (PQ and RQ) — The photosynthetic quotient is:

$$(5) \qquad PQ = \frac{\Delta O_{2_{lt}} + \Delta O_{2_{dk}}}{\Delta CO_{2_{lt}} + \Delta CO_{2_{dk}}}$$

or (absolute increment of oxygen produced in the light chamber + that consumed in the dark chamber) divided by (CO_2 consumed in the light chamber + that produced in the dark chamber). All values must be in molar units.

Respiratory quotients are calculated only from dark chambers:

$$(6) \qquad RQ = \frac{\Delta CO_{2_{dk}}}{\Delta O_{2_{dk}}}$$

Since crayfish, tadpoles, and macrobdellid leeches, which were abundant in the areas studied, were excluded from the enclosures and few other animals were seen on rocks within a chamber, it seems unlikely that macroheterotrophs affected calculated PQ or RQ significantly. Zooplankton formed an insignificant part of the community biomass. The importance of heterotrophic microbiota is unknown, but in general its inclusion would tend to lower PQ's and to have little effect on RQ's.

Results and Discussion

Seasonal Rates of Carbon Uptake

All results presented in this subsection were obtained by direct measurement of CO_2 consumption, as described above. Average daily production (mg C/m^2/day) was highest at the west station in lake 239, and lowest at the east station. The north station was intermediate (Fig. 4). During the 188 days of measurement, which approximated the average duration of an ice-free season, 456 kg C were fixed by epilithiphyton in the rocky zones of the lake (net production), an average of 2.43 kg C/day. In 1971, lake 240 epilithiphyton produced 248 kg C in 127 days. During the same period, lake 239 epilithiphyton produced 286 kg C. Assuming that production for the rest of the ice-free season was in proportion, lake 240 production would be 395 kg C, averaging 2.10 kg C/day for the ice-free season.

In spite of the large variation due to substrate heterogeneity, several generalizations appear to be possible. Average production in lake 239 in 1971 was highest at a depth of 0.1 m or 1 m, (Fig. 6) in spite of the fact that the average standing crop of epilithiphyton (measured as chlorophyll-*a*) was lowest at these depths (Fig. 5). Similar results were obtained in lake 240. Production was roughly inversely related to chlorophyll-*a* (Fig. 6 and 7).

In 1971, average daily production was highest in July, decreasing slowly through the rest of the season (Fig. 8). There was also a slight decline in chlorophyll concentration throughout the summer, followed by an increase in midfall, when the thermocline in the lake was moving rapidly downward. Production in 1972 was highest in May at shallow depths, with a continuous increase over the period of observation deeper in the water column. Together these observations suggest that production was higher earlier in the season. Nutrient deficiencies or senescing of the algae may be responsible for the decline later in the season.

Respiration and Dark Uptake of ^{14}C

Respiration, as measured by the DIC method, changed very little with season or depth. Averages are shown in Fig. 9. The overall mean was 15.6 mg C/m^2/day. Uptake of ^{14}C in the dark was negligible, with values ranging from 1 to 5% of light fixation.

Linearity in Carbon Uptake with Time

Experiments with phytoplankton have revealed that primary production is severely reduced at low concentrations of DIC (Schindler et al. 1973). To ensure that concentrations of DIC, or other nutrients, were not reduced to limiting levels during standard incubations, we sampled several times in the course of some experiments. Results were linear with time for at least the duration of our 4-hr experiments (Fig. 10A). Once CO_2 concentrations fell below 70–80 μmoles/liter there was a dramatic increase in output of CO_2. Such low concentrations were not reached in 4-hr incubations. We do not know whether oxygen uptake was similarly affected. There was no correlation between rate of uptake and light intensity (Fig. 10B), and photosynthesis at both 1 and 2 m appeared to be light-saturated on the dates tested. Some factor other than light therefore appears to be controlling production. Further tests on the relation between light and periphyton production are under way.

Chlorophyll-*a*

Typically, a positive correlation has been found between chlorophyll-*a* and production of aquatic plants (Wetzel 1964). The significant inverse relation ($\beta = -0.43 \pm 0.15$, 95%) observed in this study (Fig. 7) is therefore somewhat surprising. Since highest chlorophylls were usually found at deeper depths (Fig. 5) where chlorophyll degradation products might be expected to accumulate and the

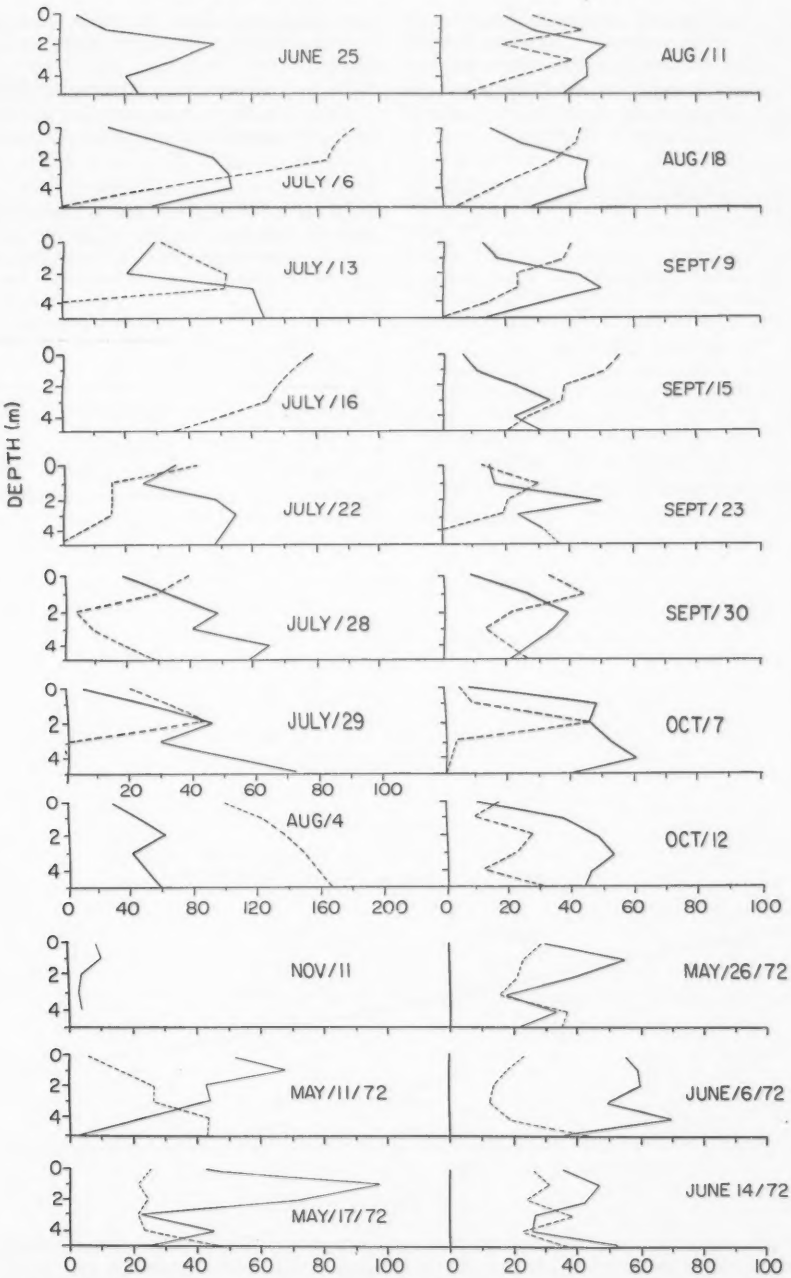

FIG. 6. Net production and chlorophyll-*a* profiles for epilithiphyton on several dates, lake 239, west station. Results from other stations and from lake 240 were similar, and are not shown. Dashed lines mgC/m²/day; solid lines mg chlorophyll-*a*/m².

chlorophyll technique used did not correct for such products, these results may be misleading. Recent tests in our laboratory indicate that standard methods for correcting for phaeo-pigments are unreliable. Proper corrections are difficult to perform on a routine basis (Dailey et al. 1973).

Differences in Results Obtained by Different Techniques

Agreement between methods was very poor although all methods showed the same general trends in production. The oxygen method usually gave the highest results, and the ^{14}C uptake method the lowest (for example Table 1).

Since some evolved oxygen might remain trapped in the algal layer due to the relatively low solubility of this gas in water (Wetzel 1965), the oxygen technique should, if anything, underestimate production. Since the precision of methods used appears to be good enough to eliminate experimental error as an explanation, the difference might be due to high photosynthetic quotients, as discussed below.

The lack of agreement between the three carbon methods is more distressing. The low values obtained by the ^{14}C uptake method may be the result of self-

Table 1. Comparisons of the four methods of measuring periphyton production done simultaneously on the same five chambers. Oxygen results are converted to carbon by assuming a PQ of 1, as is commonly done.

Method	Means and SD at two depths, mg C/m²	
	1 m	2 m
^{14}C uptake	42.6± 7.5	44.8±13.2
^{14}C disappearance	134.9±32.2	66.2±24.8
direct DIC	68.6±20.2	68.8±15.3
direct O_2	306.9±23.5	270.1±54.8

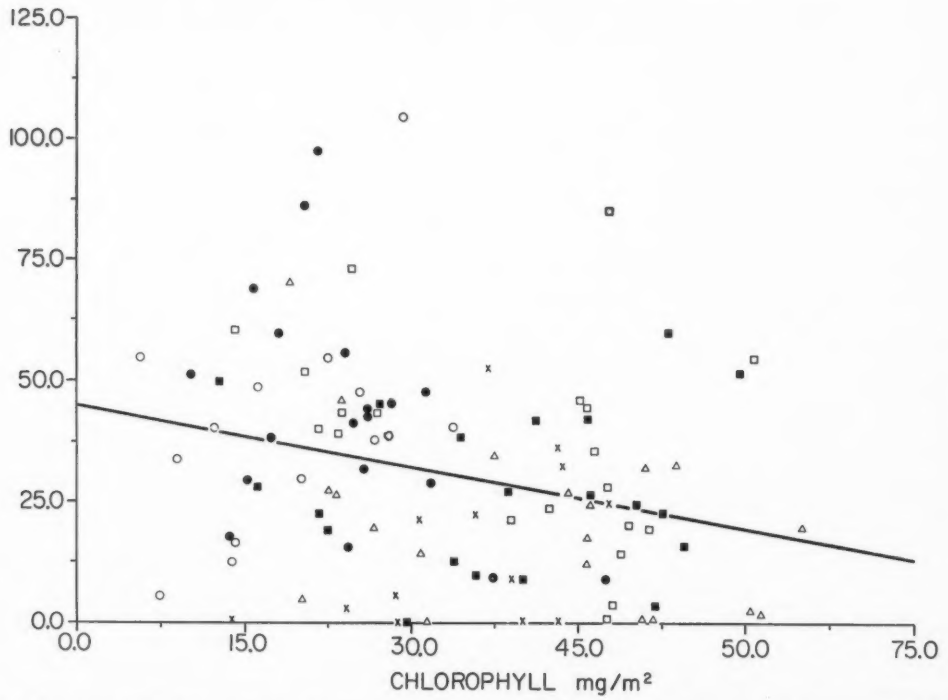

Fig. 7. The relation between net carbon uptake (Pr_n) in mg C/m² per day and chlorophyll-*a* (uncorrected for phaeophytins) for epilithiphyton in lake 239. All dates depths and are included in the analysis. ○...0.1 m; ●...1 m; □...2 m; ■...3 m; △...4 m; x...5 m. The slope of the regression line is –0.43 ± 0.15 (95% confidence limits).

absorption during counting, since some particulate matter (chiefly small mineral particles) remained in the scintillation vials, even after digestion with NCS. Cell breakage during scrubbing of rocks could also have caused loss of particulate ^{14}C. One would, however, expect good agreement between the ^{14}C disappearance and direct CO_2 methods, as is observed at 2 m depth in Table 1 if the ^{14}C technique measures net production. This leaves us somewhat at a loss to explain the results at 1 m. It is possible that excretion of dissolved organic products of photosynthesis could cause the difference; since the "^{14}C disappearance" method did not distinguish between organic and inorganic forms of dissolved carbon, it would not include extracellular products. Wetzel (1964) found that aquatic macrophytes excreted up to 10% of the total carbon fixed in photosynthesis. Although more experimental work is clearly required, results show that comparisons between lakes where production has been measured by different techniques must be made with caution.

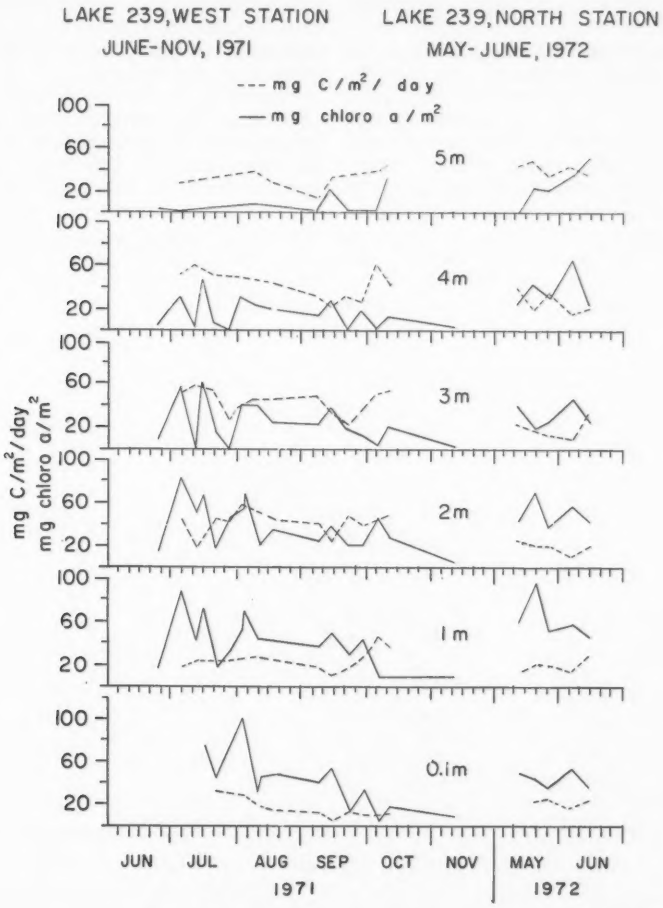

FIG. 8. Average daily net production and standing crop of epilithiphyton in the littoral zone of lake 239, west station, June–November 1971, and north station, May–June 1972. Seasonal trends at other stations and in lake 240 were similar.

Photosynthetic and Respiratory Quotients

Photosynthetic quotients recorded during the season averaged 2.6 with a standard deviation of 1.7 (all depths). Although there are no values to compare with in the periphyton literature, these results are very high when compared with phytoplankton and other plants (reviewed by Strickland 1960). In culture studies where nitrate is the main nitrogen source, PQ's as high as 1.5 have been found. It has been hypothesized that under such circumstances NO_3 and CO_2 compete for reduction by a limited amount of radiant energy (Strickland 1960).

There has been no previous study of the effect of extremely low CO_2 concentrations on either CO_2 uptake or O_2 evolution by periphytic algae. In preliminary investigations of phytoplankton production at low DIC concentrations we have not found abnormally high PQ's. At high light levels and with an excess of CO_2, van Niel et al. (1953) found an increase in PQ. Cramer and Myers (1948) hypothesized that nutrient starvation results in low PQ, and they (Myers and Cramer 1948) believed that the high PQ's which they found for algae exposed to low light after prolonged periods of high illumination were due to synthesis of protein by carbohydrate-glutted cells. Ammann and Lynch (1965, 1966, 1967) found that varying the available oxygen, CO_2, and intensity and wavelength of the light source did not alter the photosynthetic quotient of *Chlorella pyrenoidosa* to any significant degree. Their DIC concentrations were, however, far higher than in our lakes. The lack of physiological data for natural populations of algae, and for periphyton in particular, severely limits the interpretation of results.

Respiratory quotients were also higher than expected, with a seasonal mean of 1.1 ± 0.9. These are not unusual for algae; values as high as 1.6 have been recorded from *Chlorella* cultures when nitrate is the main nitrogen source. A few

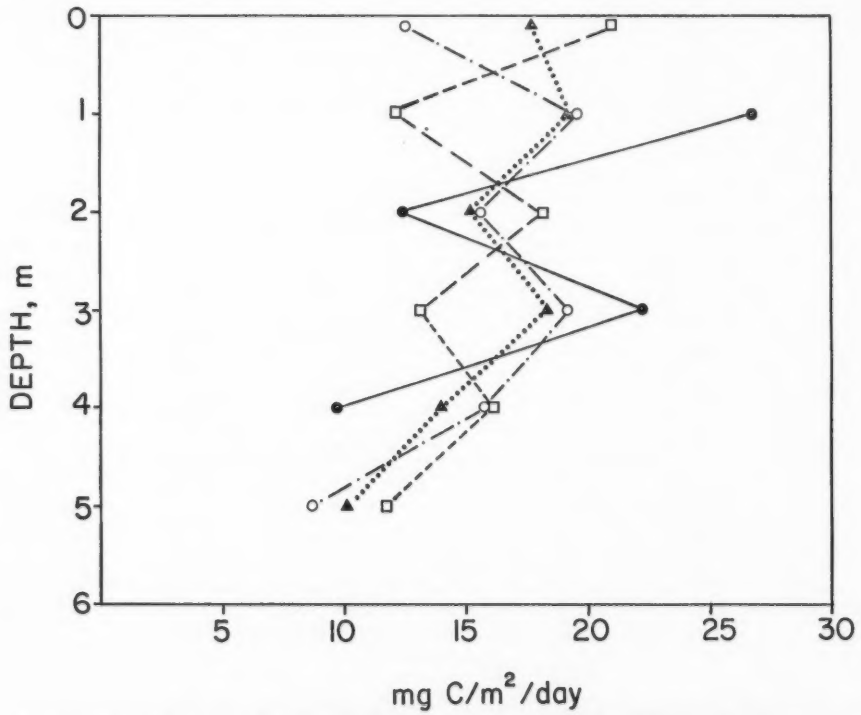

FIG. 9. Average respiration in dark chambers, measured by the DIC technique, 1971. The overall mean for all depths was 15.6 mg C/m²/day with a standard deviation of ±3.3. ●——● east station; □---□ north station; ○·–·○ west station; ▲....▲ average for all stations.

even higher values have been recorded for phytoplankton (Strickland 1960).

One possible explanation for the "physiologically impossible" PQ's is that there is some sort of diel asynchrony in the uptake of CO_2 versus release of oxygen by periphyton, i.e. oxygen release may have been lower or CO_2 uptake higher at low light intensities. All of our measurements were made at midday, therefore it is impossible to give even a preliminary test to this hypothesis at present. Further testing is in progress.

Comparison with Results from Other Studies

Only three other studies of periphyton production on natural substrates are detailed enough to make comparisons worthwhile. Both Borax Lake (Wetzel 1964) and Marion Lake (Hargrave 1969, Gruendling 1971) are extremely shallow ($\bar{z}$ = less than 0.5 and 2.2 m, respectively, compared to 10.5 and 6.1 m for lakes 239 and 240); so that periphyton growth covers the whole lake bottom. Borax Lake has a pebbly bottom, while that of Marion Lake is of soft ooze. Lawrence Lake (Allen 1971, Rich et al. 1971) is a marl lake with extensive growths of macrophytes. The O_2 method was used on Marion Lake, and ^{14}C on the others. Our comparisons of methods indicate that comparing the results from these studies may be difficult; however, because the lakes are of extremely divergent character some interesting information of a general nature should result.

All of the lakes studied except Marion Lake had very similar phytoplankton production rates (Table 2). One must remember that the acidification and bubbling technique (Schindler et al. 1972) was used for phytoplankton on lakes 239 and 240, so that results obtained will be somewhat higher than from the unmodified ^{14}C filtration technique.

The greatest differences in production were in periphyton algae. These plants contributed nearly two orders of magnitude less to production in lakes 239 and 240 than in other systems. Even when comparisons are restricted to similar depths, i.e. the 0–1-m zone in lakes 239 and 240 is compared to other lakes, production per unit substrate area is far lower in our lakes. A combination of factors, including substrate suitability, nutrient availability, and climate, is probably responsible. Preliminary tests indicate that nonrocky littoral areas in lakes 239 and 240 support little periphyton production, since they are often covered with flocculent al-

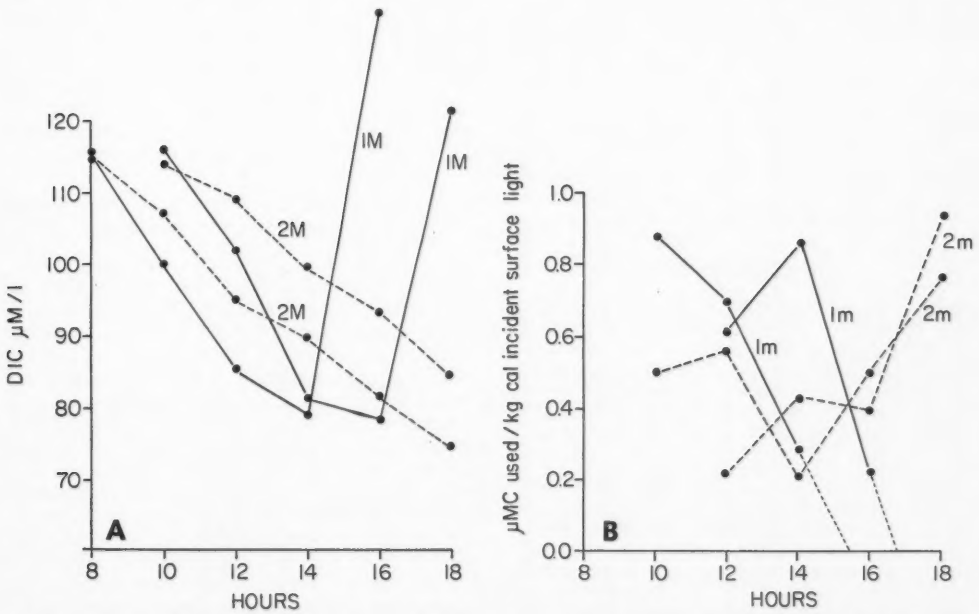

FIG. 10. Results of extended incubation tests with epilithiphyton. A) DIC concentration with time; B) DIC uptake per unit light per unit time.

TABLE 2. Annual net production by aquatic plant communities in several freshwater lakes. Only studies in which production estimates were for periphyton on natural substrates are included.

Author	Lake	Method	A ha	$\bar{z}$ m	Lake type	Type of periphyton community	gC/m² of substrate surface/year	gC/m² of substrate surface/year	kgC/ha of lake surface/year		
									Phytoplankton	Periphyton	Macrophytes
Wetzel 1964	Borax	^{14}C	39.8	<0.5	saline	primarily epilithic, some epiphytic	267	–	926[a]	692[c]	12
Hargrave 1969 Efford 1967	Marion	O_2	13.3	2.2	softwater oligo.	epipelic	31	same	8[c]	310[c]	180
Rich et al. 1971	Lawrence	^{14}C	5.0	5.9	marl	epiphytic on dense macrophytes and epilithic		–	507	380	879
	lake 239	CO_2	56.1	10.5	softwater oligo.	epilithic	5.19	7.29	823[d]	8.1	unmeasured[b]
	lake 240	CO_2	44.1	6.1	softwater oligo.	epilithic	5.18	7.25	501[d]	9.0	unmeasured[b]

[a]Wetzel's stated average daily figure × 365 ÷ 39.8 ha.
[b]Likely to be insignificant, since macrophytes are poorly represented.
[c]Wetzel's total annual mean kg C/lake day × 365 days ÷ 39.8 ha, the average area of the lake.
[d]Based on 3 year's data. Winter production is not included.
[e]Calculated from figures for gross production and algae respiration from Hargrave's Figure 13.

lochthonous material from surrounding forests. This, added to the larger size and greater depth of our lakes, also contributes to the low ratio of periphyton production to phytoplankton production in lakes 239 and 240.

Macrophytes were undoubtedly far less productive in lakes 239 and 240 than in other systems. The group is very poorly represented in both lakes, with small areas of *Lobelia dortmanna* and *Eriocaulon septangulare* appearing in July. Larger plants, including *Potamogeton amplifolius*, *Typha latifolia*, and species of *Sagittaria*, *Scirpus*, *Isoetes*, and *Utricularia*, can be literally counted on the fingers in both lakes. The scarcity of macrophytes in ELA lakes may be due to the low concentrations of major cations and anions which are usually necessary for successful growth of angiosperms in the lakes. Armstrong and Schindler (1971) give representative chemical data.

Lakes 239 and 240 have larger areas of suitable substrate for epilithiphyton and more macrophytes than almost all of the lakes controlled by the Experimental Lakes Program. The sole exception is probably lake 305, which has extremely clear waters and large areas of algae-covered boulders extending to a depth of 20 m. The lakes used in fertilization experiments so far (lakes 227, 304, 302, 226, 261) have waters more highly stained and richer in phytoplankton than lakes 239 and 240, so that light penetration to the bottom is limited to small areas lining the shores. Their bottoms are also largely of flocculent organic material, with only small areas of rock suitable for epilithiphyton. Primary production studies in these lakes have therefore concentrated upon planktonic algae.

Significance of Results to Other Studies at ELA and Elsewhere in Precambrian Shield

Extremely high "turnover" of nutrients would be necessary to make the small epilithiphyton communities of ELA lakes important contributors to lake metabolism. Our data indicate that this is not the case. In both lakes 239 and 240, epilithiphyton utilize two orders of magnitude less carbon than phytoplankton. Preliminary experiments with radiophosphorus, both in lake 239 (D. R. S. Lean and D. W. Schindler unpublished data) and in isolated columns in lake 227, indicate that the contribution of epilithiphyton to overall lake dynamics of this element is small. It therefore appears that the early decision to concentrate most effort in fertilized lakes at ELA on planktonic communities was a valid one. In larger, deeper lakes, where a higher proportion of the bottom lies below the photosynthetic compensation depth, the community would play an even less important role. Such evaluations of the relative roles of epilithiphyton, macrophyte, and phytoplankton communities are critically needed for a variety of lake types in various geological substrates, so that efforts to predict nutrient dynamics may be economically directed.

Conclusions

The extreme heterogeneity of substrates poses one of the major difficulties in dealing with periphyton communities. Although the enclosures used in our studies are far larger than those used by other workers, still larger ones appear to be necessary in order to reduce substrate variability to the point where accurate interpretations of factors affecting periphyton production can be made.

Superimposed upon the above difficulty is the variable and unpredictable natural light regime, which renders the comparison of results obtained on different days nearly impossible. Fee (1973) has demonstrated that such difficulties may pose serious errors when attempting to extrapolate from short in situ incubations to seasonal estimates of phytoplankton production. Similar experiments with epilithiphyton are currently under way.

The great differences in results of different methods also makes comparisons between studies very difficult, as we have demonstrated above, although all appear to show similar trends and to have acceptable levels of precision.

The above combination of problems makes it appear extremely unlikely that in situ measurements of periphyton production made by current techniques will allow precise predictions about the functioning of periphyton communities. Future research should concentrate on new approaches to the measurement of periphyton production.

Acknowledgments

E. Fee, B. West, and N. Scott assisted with the large amount of SCUBA diving required. E. Zurbrigg performed many of the DIC analyses. M. Hendrickson scrubbed algae from rocks, and G. Jacobsen performed chlorophyll analyses. E. J. Fee, A. L. Hamilton, K. Patalas, and D. R. S. Lean provided valuable criticism of the manuscript. Figures were drawn by C. Boyce.

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Movements of Phosphorus Between its Biologically Important Forms in Lake Water

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LEAN, D. R. S. 1973. Movements of phosphorus between its biologically important forms in lake water. J. Fish. Res. Board Can. 30: 1525–1536.

A model consistent with the kinetics of phosphorus in epilimnetic lake water was developed. Adding $^{32}PO_4$ to lake water and separating the major forms of dissolved phosphorus by Sephadex gel filtration showed that the exchange mechanism between inorganic phosphate and the particulate fraction predominates. At the same time, a low-molecular-weight phosphorus compound is excreted which combines with colloids in lake water, releasing phosphate from the colloid and making the phosphate available for "transfer" again. This rapid cycling of phosphorus between the four principal forms — the particulate fraction, the low-molecular-weight P compound, colloidal P, and phosphate — appears to contribute to formation of colloids in lake water. No direct complexing of phosphate to the colloid was observed. Only in the presence of algae, bacteria, and other particulate matter did the radioactive phosphorus move to the low-molecular weight and the colloidal forms. The low-molecular-weight compound is negatively charged, as is the colloidal P, but to a lesser degree. Both are removed by anion exchange materials along with phosphate, but the rate that they move into the fraction removed by membrane filtration is different from that for phosphate. When filtrate is refiltered a large amount of the colloidal P is retained by the filter. This complicates measurements of transfer and makes previous studies on utilization of dissolved organic phosphorus of doubtful value since corrections for filter retention were rarely, if ever, made.

LEAN, D. R. S. 1973. Movements of phosphorus between its biologically important forms in lake water. J. Fish. Res. Board Can. 30: 1525–1536.

On a développé un modèle qui s'accorde avec le mouvement du phosphore dans l'eau de lac. L'addition de $^{32}PO_4$ à l'eau de lac et la séparation, par filtrattation sur gel Sephadex, des principales formes de phosphore dissous démontrent que le mécanisme d'échange entre le phosphate inorganique et la fraction particulaire prédomine. En même temps, il y a excrétion d'un composé phosphorique de faible poids moléculaire, qui se combine avec les colloïdes dans l'eau de lac, libérant le phosphate du colloïde et rendant le phosphate à nouveau disponible pour "transfert." Ce cyclage rapide du phosphore entre les quatre formes principales — la fraction particulaire, le composé de P de faible poids moléculaire, le P colloïdal et le phosphate — semble contribuer à la formation des colloïdes dans l'eau de lac. On n'observe pas de complexation directe entre le phosphate et le colloïde. Ce n'est qu'en présence d'algues, de bactéries et autre matière particulaire que le phosphore radioactif se déplace vers les formes de faible poids moléculaire et colloïdales. Le composé de faible poids moléculaire a une charge négative. Il en est ainsi du phosphore colloïdal, mais à un degré moindre. Les deux sont absorbés par des matériaux d'échange d'anion en même temps que le phosphate, mais la vitesse à laquelle ils pénètrent dans la fraction enlevée par filtration sur membrane diffère de celle observée pour le phosphate. Quand on filtre de nouveau le filtrat, le filtre retient une grande quantité de P colloïdal. Ceci complique les mesures de transfert et tend à dévaloriser les études antérieures sur l'utilisation du phosphore organique dissous, car one ne fait que rarement ou jamais les corrections qui tiennent compte de cette rétention.

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ENRICHMENT experiments have demonstrated that phosphorus additions to lakes can increase the standing crop of phytoplankton (e.g. Schindler et al. (1971, 1973). Yet no overall model exists which relates phosphorus loading to the changes in lake metabolism. To do so requires identification of the biologically important phosphorus forms and measurements of the flux rate between them.

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This paper presents part of a study intended to provide a model for phosphorus dynamics within the epilimnion of lakes.

The kinetics[1] of $^{32}PO_4$ "transfer"[1] have been studied by Rigler (1956, 1964), who showed that phosphate was rapidly exchanged between the particulate and dissolved fractions in lake waters and proposed a two-compartment exchange model to explain his observations. Chamberlain (1968), however, demonstrated kinetics that were occasionally diphasic, so that a more complicated model was required. To fit with these observations, Rigler (1968) proposed that phosphate complexed with an unidentified substance in lake water. This hypothesis was tested by Lean and Rigler (1973) and rejected. Instead, they showed that the seston was responsible for formation of a nonanionic filtrate ^{32}P form.

The experiments using $^{32}PO_4$ neglect the great quantity of phosphorus in the soluble unreactive phosphorus (SUP) pool. This could be an important source of phosphorus for algae (Rigler 1964). It would seem that we must subdivide the SUP pool and measure the biologically important forms, together with rate constants, for their formation and transfer.

Chamberlain (1968), in a preliminary investigation of the nature and importance of soluble organic phosphorus in the phosphorus cycle of lakes, used Dowex 1-X8 anion exchange resin to characterize the ^{32}P-labelled filtrate fractions in lake water which had been incubated with $^{32}PO_4$. Although his columns removed $^{32}PO_4$ at almost 100% efficiency, only about 80% of the filtrate ^{32}P was removed. The compounds retained eluted like $^{32}PO_4$. However, he also showed that the uptake rates by the fraction removed by membrane filtration were different for filtered ^{32}P and $^{32}PO_4$. Filtrate ^{32}P was taken up more slowly and the data points did not extrapolate to 0% utilized at time zero. He termed this "instantaneous uptake" or "refilterable solids." This inconsistency suggests that ion exchange techniques inadequately characterize filtrate ^{32}P.

In this study, $^{32}PO_4$ was added to whole and filtered lake water, and Sephadex gel filtration was used for distinguishing the labelled filtrate fractions at various intervals after $^{32}PO_4$ was added. With the Sephadex technique, strong reagents are not required, so that possible hydrolysis of labile organic compounds is minimized. Concentration of the filtrate is unnecessary since only the radioactivities of the various fractions are measured. Molecules are separated according to size, and the time course of the appearance of radioactivity in the various forms can be obtained. Extremely small amounts of each labelled fraction can then be used in subsequent experiments to measure transfer rates, filter retention and ion exchange properties, and abiotic changes such as complexing and hydrolysis.

These experiments on many dates from June to September 1970, led to a model for phosphorus dynamics which has been briefly introduced by Lean (1973a). Though transfer curves obtained were never the same on any 2 days, their consistent pattern led to development of a model for phosphorus movement in epilimnetic water. All the data are given in Lean (1973b), and selected data presented here to illustrate the major observations, along with some improvements in the model.

Materials and Methods

"Transfer" curves were obtained as described by Lean (1973a, b,) and Lean and Rigler (1973). Water was collected by pooling subsamples of the epilimnion of Heart Lake, a small eutrophic lake 30 miles northwest of Toronto, Ont. $^{32}PO_4$ was added to 400–600 ml within 2 hr after sampling and 5-ml aliquots were withdrawn after tracer addition for membrane filtration using 0.45-μ Millipore® filters. Concurrently, the ^{32}P-labelled compounds in the filtrate were distinguished from $^{32}PO_4$ using Sephadex gel filtration chromatography. Columns of Sephadex G25, G75, and Sepharose 4B, were prepared as outlined in the Pharmacia® technical literature.

Four columns of G25 with bed dimensions of 2.5 by 32–35 cm, were used so that a sample could be injected in each over the first hour of experiment. Samples (2.5 ml) of filtrate were injected into the column, then eluant (0.3% sodium chloride and 0.02% sodium azide) was pumped through the columns using a Buchler peristalic pump. The flow rate was 0.5 to 1.0 ml/min. Five-millimeter samples were collected using a Buchler Fractionomat and a Gilson fraction collector. When all samples had been collected, 0.5 ml of each was plated on aluminum planchets and the radioactivity measured using a Nuclear Chicago gas flow counter. No irreversible binding of the radioisotope to the Sephadex material was found.

The Sephadex G-25 columns were used to fractionate a molecular size range of approximately 100–5000. A G-75 (5 cm by 35 cm) column was also prepared, and 5–20-ml samples were injected. This was used for molecular weights up to 70,000. The eluant, as above for G-25 columns, flowed by gravity from a mariotte flask and fractions were collected manually. A Sepharose 4B column was prepared to the same bed dimensions as the G-25 columns and operated in the same way. This separated compounds of molecular weights (for polysaccharides) up to 5,000,000. The columns were calibrated using Blue Dextran to determine void volume and $^{32}PO_4$ to determine the elution volume for PO_4.

[1]The term "transfer" is used in this paper instead of the more commonly used terms "uptake" and "utilization" since little evidence exists that the fractions removed by filtration are actually taken up or utilized.

Experiments similar to those described by Chamberlain (1968) were conducted to compare the transfer rates for filtrate ^{32}P and $^{32}PO_4$. Filtrate ^{32}P (5 ml) from lake water which had been incubated wtih $^{32}PO_4$ was added to 100 ml of whole lake water and the transfer rate compared with that for $^{32}PO_4$ added to 100 ml of whole lake water. This provided a measure of the biological activity of the labelled filtrate phosphorus fractions.

To determine whether the instantaneous "uptake" of ^{32}P ("refilterable solids") observed by Chamberlain (1968) represented filter retention rather than complexing with a sestonic compartment, filtrate ^{32}P was added to filtered lake water and passed through another 0.45-μ filter. Following the 0.45-μ filtration, samples were passed through a Gelman® 6404 or 6407 anion or a cation exchange filter. In this way, the ionic properties of the filtrate ^{32}P forms were determined and compared to the gel filtration analysis for both the $^{32}PO_4$ added to the filtered lake water and ^{32}P from lake water incubated with $^{32}PO_4$. The fractions of filtrate ^{32}P obtained by gel filtration were also added to filtered lake water and the efficiency of removal measured by ion exchange. This was intended to show if the filtrate ^{32}P forms had ion exchange properties even though they were not $^{32}PO_4$. The ion exchange filters were 47 mm in diameter and had an effective filtering diameter of 35 mm. In the uptake experiments, 5 ml was passed through a 25-mm filter with an effective filtering diameter of 20 mm. Since the effect of volume filtered was thought to be important, the corresponding volume per unit surface area to the 25-mm filters was used, i.e. $((35)^2/(20)^2 \times 5$, or 15.3). The volume used in these experiments was 15 ml. Flow rates were held constant at 40–60 ml/min.

Formation of Nonorthophosphate Compounds

Throughout the summer period, the transfer of $^{32}PO_4$ was always rapid. Fifty percent was usually taken up in less than 2 min. The kinetics observed were usually diphasic rather than consistent with a two-compartment exchange model (Lean and Rigler 1973).

On June 11, the initial transfer rate was very fast, followed by a slower further transfer to an approximate asymptote of 4%, (Fig. 1). G-25 Sephadex analysis, even at 5 min, indicated three radioactive forms (Table 1). Peak 1 corresponded to void volume, i.e. exceeded the fractionation range for G25 (5000 MW). Peak 2 had an elution volume corresponding to an approximate molecular size of 250, while Peak 3 corresponded to $^{32}PO_4$. The $^{32}PO_4$ peak was initially large but decreased rapidly and at 88 min half of the ^{32}P in the filtrate was in this form. A maximum was reached in the activity of the peak 2 (250 MW) form at the 5- and 18-min samplings whereas the maximum for the peak 1 (high MW) form occurred at the 46-min sampling (Table 1).

When the data points for transfer of filtrate ^{32}P (Fig. 1) were extrapolated to zero time, there was a 28% "instantaneous uptake" followed by a transfer much slower than that for $^{32}PO_4$. This shows that the forms of ^{32}P in the filtrate cause the transfer rate to differ from that expected for $^{32}PO_4$.

On June 21, similar experiments were conducted (Fig. 1), plus others which contributed to a better understanding of the formation of the large molecular weight form, its size, and the factors which influence the shape of the transfer curves, After analyses of the filtrate fractions (Table 1). the filtrate obtained after 120-min incubation with $^{32}PO_4$ was injected into a G-75 Sephadex column (MW range 5000–70,000). Only two major peaks were observed, one at void volume, i.e. greater than 70,000, and one for low MW fraction (Fig. 2). The fraction which exceeded the fractionation range for G25 is also excluded from G75.

The possibility that the large molecular weight fraction was formed by direct complexing of $^{32}PO_3$ with an abiotic material was investigated by adding $^{32}PO_4$ to filtered lake water and after 45 min injecting a sample into G-25 sephadex. Results from this experiment were similar to

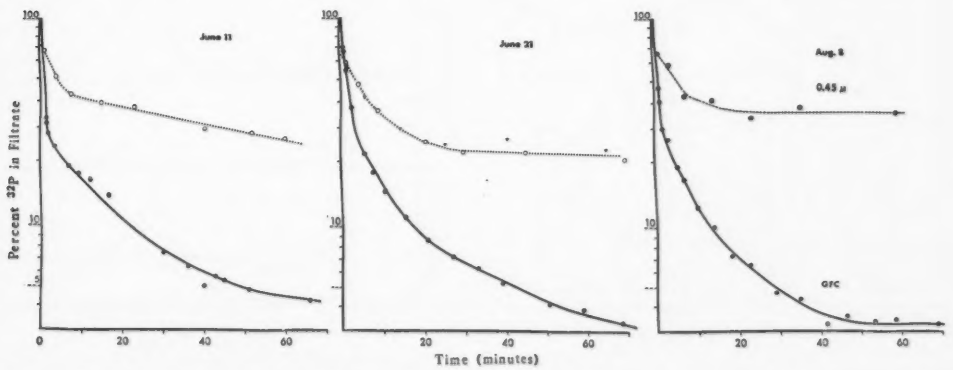

FIG. 1. Percentages of ^{32}P in filtrate (●) at various intervals after $^{32}PO_4$ was added to whole lake water on three dates (0.45-μ Millipore and Whatman GFC filters used on August 8); and filtrate "transfer" curves obtained on first two dates using filtrate from lake water incubated with $^{32}PO_4$ for 30 min (on June 21, 50 ml filtrate added to 50 ml whole lake water (▲) and 10 ml to 90 ml (○)). For Sephadex analyses see Table 1 and Fig. 2.

those conducted on June 11, July 16, August 6, and August 11. No detectable amount of $^{32}PO_4$ moved to the peak 1 position. This clearly shows that the formation of peaks 1 and 2 involves the seston and confirms Lean and Rigler's (1973) conclusion that direct complexing of $^{32}PO_4$ to substances in lake water occurs rarely and cannot be used to explain diphasic uptake curves.

The filtrate ^{32}P transfer experiment (Fig. 1) differed from the others in amount of filtrate added. After unfiltered lake water was incubated with $^{32}PO_4$, 50 ml of filtrate ^{32}P was added to 50 ml of whole lake water and the curve compared to that for 10 ml of filtrate ^{32}P added to 90 ml of whole lake water. Both curves were similar. The instantaneous transfer appeared to be 30% followed by a further uptake of 50%. One would suppose that the lower biomass combined with a higher PO_4 concentration, due to possible cell damage, would result in a slower transfer. This was not found; instead, some other rate-controlling mechanism must be involved.

The next step was to determine the stability of the various forms of filtrate ^{32}P. The distribution of the radioisotope in the various fractions present in a sample at 40 min after incubation with $^{32}PO_4$ was determined in the usual way. Half of the injected sample was kept in a sterile test tube and injected 4 hr later (Fig. 2). Peak 2 disappeared, peak 1 decreased slightly, and the inorganic peak increased by a corresponding amount. In other words, the peak 2 form is unstable and, after filtration, moves to the PO_4 position. This observation was important not only in the development of the model for phosphorus dynamics but must be considered before a reliable test to measure orthophosphate can be found.

The transfer curve for the July 27 experiment (Table 1) has been reported earlier, (Lean 1973a). It was similar to those shown for June 11 and 21.

On July 28, 15 min filtrate was injected into a G-75 column. The result was the same as on June 21. Two major peaks were observed, one at void volume and the other at the elution volume for $^{32}PO_4$ and other

TABLE 1. Percentages[a] of radioactivities that were found in three phosphorus fractions in "transfer" experiments on five dates in 1970. Peak 1 represents the fraction appearing at void volume (Vo) and peak 2 has an elution volume corresponding to 250 MW.

Time (*min*)[b]	Peak 1	Peak 2	$^{32}PO_4$
	June 11		
5	1.0	1.8	18.2
18	1.3	1.8	7.9
46	2.0	1.4	1.8
88	1.1	0.4	1.5
	June 21		
2.5	0.9	0.7	36.4
15	1.3	1.3	8.9
40	1.1	0.6	3.3
180	1.0	0.5	0.5
	July 27		
3	0.7	0.8	19.5
15	0.9	1.3	5.3
53	0.8	0.4	0.8
	July 28, 11.1 min filtrate		
5	8.6	10.7	80.5
25	10.1	9.4	80.6
55	8.0	6.9	85.1
155	9.3	3.0	87.7
	Aug.11, 55 min filtrate		
2	24.6	13.5	61.9
15	21.2	8.1	70.6
55	20.2	4.0	75.8
330	16.0	2.3	81.7

[a]Of totals on first three dates and of activities in filtrate on last two.
[b]For first three dates, after addition of $^{32}PO_4$; for last two, after filtration.

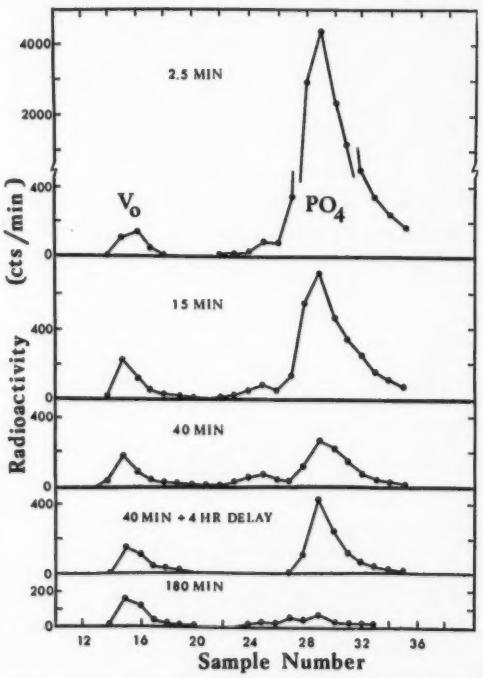

FIG. 2. Radioactivities of filtrate fractions at various intervals, June 21. The 40-min filtrate was reinjected after 4 hr. V_o, void volume for G-25 Sephadex; PO_4, elution volume for phosphate.

small phosphorus molecules. A Sepharose 4B column was also prepared which extends the range to a molecular weight of 20,000,000 for proteins and peptides and 5,000,000 for polysaccharides. Filtrate from lake water incubated with $^{32}PO_4$ was injected into this column. The peak 1 form still appeared at void volume; therefore, it is even larger than 5,000,000 or it is simply excluded by the sephadex material, i.e. it is a small molecule but does not penetrate the pores.

To test this possibility, peak 1 material was collected and layered on a sucrose density gradient (McConkey 1968), of 27-ml 5–20% sucrose, and centrifuged for 17 hr at 23,000 rpm, using a S.W. 25.1 rotor (average force 57,000 g) at a temperature of 4 C. Following ultracentrifugation, the gradients were collected in 1-ml fractions using an Isco Model D Density Gradient Fractionator. The radioactivity was determined for each sample. Calibration and other operating procedures are given by Hermolin (1971).

Some activity was observed in the 10S range but at least 68% of the radioactivity exceeded 60S, the separation range of the gradient (Fig. 3). These results confirm that the peak 1 material is of large molecular weight material or an aggregate and is referred to as colloidal ^{32}P in the discussion.

It was fortuitous that only three major forms were found: colloidal P (Peak 1), Peak 2 (approximate MW 250), and phosphate. The peak 2 material will simply be referred to as XP. Further experiments contributed toward an understanding of the interrelationships of these three forms.

Earlier, on June 21, it was shown that abiotic changes took place in the relative proportions of the three major filtrate ^{32}P forms. On July 28, this phenomenon was more carefully investigated using filtrate from lake water incubated with $^{32}PO_4$ for 11.1 min. On August 11, a similar experiment was conducted using 55-min filtrate (Table 1 and Fig. 4). The August 11 experiment was briefly reported earlier (Lean 1972). In both experiments, the peak 2 (XP) form decrease until it disappeared, colloidal ^{32}P also decreased slightly, and the $^{32}PO_4$ peak increased by an equivalent amount.

This change was shown to influence the shape of the filtrate transfer curves. On July 28, half of a sample of filtrate ^{32}P, obtained from a sample of lake water incubated with $^{32}PO_4$ for 36 min, was immediately added to a sample of lake water and the transfer rate compared to that for the other half of the sample, which was kept in a sterile test tube for 150 min and then added to lake water. (Fig. 5) The two curves were similar in that there was an instantaneous transfer of about 30% but the transfer curve for the sample kept in the sterile test tube for 150 min after filtration was more rapid. The two approached similar asymptotes much higher than that for $^{32}PO_4$. These experiments clearly point out that colloidal P and XP are responsible for the diphasic kinetics. Neither is produced in the absence of seston.

A similar experiment was conducted on August 11, using filtrate ^{32}P from lake water incubated with $^{32}PO_4$

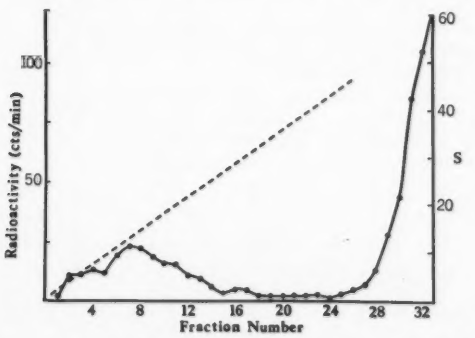

FIG. 3. Radioactivities (●) of peak 1 material, July 28, after sucrose gradient ultracentrifugation from Sephadex G-25 separations (smaller molecules were found in fractions near the top (1–10) but most were so large that they went to the bottom) and calibrated S values.

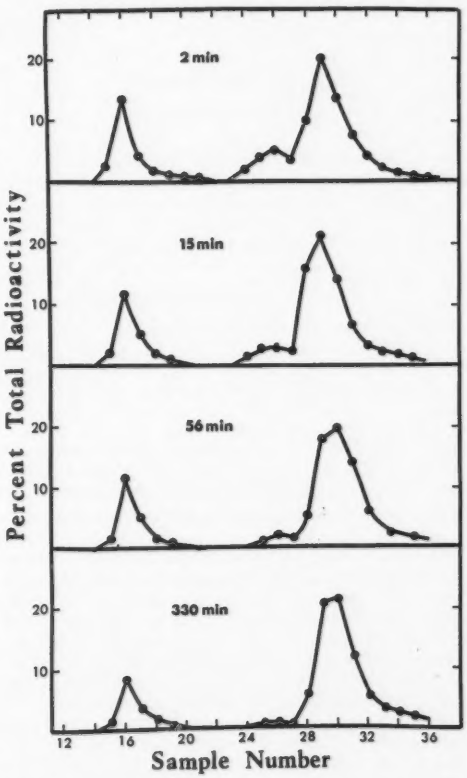

FIG. 4. Percentages of total radioactivities injected at four intervals after filtration at 55 min (August 11, Table 1) that were found in the several fractions (radioactivity shifted to $^{32}PO_4$ peak).

for 55 min. The transfer curve soon after filtration was compared to that 300 min later (Fig. 5). Sephadex analyses of the changes (Fig. 4 and Table 1) show that some of the colloidal ^{32}P and all the $X^{32}P$ shifted to the $^{32}PO_4$ form during this delay period. The transfer curve reflected this shift in that the transfer rate was faster.

Ion Exchange and Millipore Filtration Artifacts

Comparison of the "transfer" rates of filtrate ^{32}P and to $^{32}PO_4$ shows that the two nonorthophosphate fractions affect the transfer kinetics. Two factors complicate these experiments. The transfer of $^{32}PO_4$ is so rapid that often over 50% is transferred before a sample can be filtered, yet the curve is drawn to 100%. The filtrate ^{32}P transfer curves, on the other hand, do not extrapolate to 100%. The following experiments provide the justification for this practice and corrections made for filtration artifacts. At the same time, ion exchange studies were related to Sephadex gel filtration analyses allowing explanation of the earlier paradox of Chamberlain (1968), that most of the filtrate ^{32}P eluted like $^{32}PO_4$ on anion exchange columns but was utilized at a rate different from that for $^{32}PO_4$.

Filtrate ^{32}P was obtained from lake water incubated with $^{32}PO_4$ for specified periods then refiltered through a millipore filter, followed by either a Gelman anion or a cation exchange filter. As a control, $^{32}PO_4$ was added to filtered lake water and the same procedure followed. The retention of the isotope by the filter on refiltration was measured along with the ionic properties of filtrate ^{32}P.

When $^{32}PO_4$ was added to filtered lake water, no significant retention by the millipore filter was found (Table 2). This means that joining the first data point on the curve to 100% is justified. As mentioned earlier, Sephadex analyses were also conducted and no complexing of $^{32}PO_4$ in filtered lake water was found.

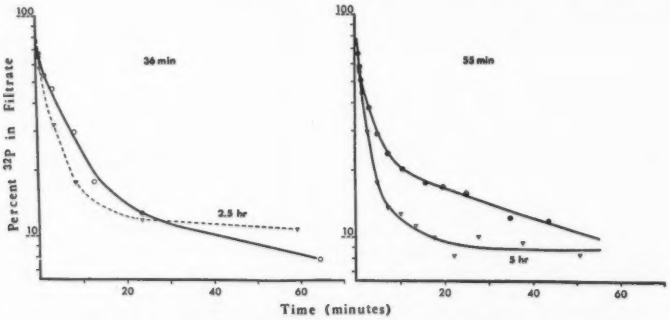

FIG. 5. Transfer curves for 36-min (July 28) (*left*) and 55-min (August 11) (*right*) Table 1; Fig. 4) filtrates immediately after filtration (○, ●) and after delay (▽).

TABLE 2. Percentages of total radioactivities (averages of at least four analyses at 0–30 min after filtration) obtained when filtrate ^{32}P and, as a control, $^{32}PO_4$ added to filtered lake water (FLW) were passed through two pairs of filters.

Date	Experiment	Millipore retention	Anionic removal	Millipore retention	Cationic removal
June 11	$^{32}PO_4$ + FLW	0.8	75.4	2.1	0.6
June 21	$^{32}PO_4$ + FLW	0.7	77.5	2.0	0.7
July 2	60-min filtrate	16.2	60.6	13.8	4.2
July 2	$^{32}PO_4$ + FLW	0.3	81.2	2.0	0.6
July 15	60-min filtrate	30.8	50.5	25.7	7.7
July 16	$^{32}PO_4$ + FLW	0.9	83.1	2.5	0.6
July 27	75-min filtrate	32.6	44.1	28.1	7.7
Aug. 6	Peak 1 + FLW	60	19	–	–
Aug. 13	Peak 1 + FLW	46	38	49	15
	Peak 2 + FLW	6	72	13	2
	Peak 3 + FLW	0.4	74.4	–	–
Aug. 16	150-min filtrate	44.8	27.1	37	11
Aug. 16	$^{32}PO_4$ + FLW	1.0	78.9	–	–

The retention on refiltration of filtrate ^{32}P by the millipore filter occasionally was as great as 46%. This approximates the "instantaneous uptake" of filtrate ^{32}P in the transfer experiments and clearly points out that this is not biological utilization.

Some material was formed, only in the presence of seston, which causes this filter retention artifact. This raises yet another question: to what extent did the initial filtration remove some of this fraction? On August 13, Whatman GF/C filters were used for the transfer experiments and compared to that for the 0.45-μ millipore (Fig. 1). The curves are considerably different. The asymptote using the glass filter is at about 32% and that for millipore at about 3%. The high values for the GF/C filtrate can be explained by postulating that the phenomenon responsible for filter retention is less important or that microorganisms are not completely filtered out. Without a complete understanding of what fractions are removed by filtration, we must conclude that techniques must be carefully specified. since filtration using different filters, vacuums, and sample sizes may give different results.

Also on August 13, peak 1 materials were obtained from a sample of Heart Lake water that had been incubated with $^{32}PO_4$ for 18 min. At this time, 22.8% of the added ^{32}P was not retained by the 0.45-μ filter. The filtrate fractions were obtained by injecting two samples of 2.5 ml each into two carefully calibrated G25 columns. In this way, 10 ml of each isolated peak material was obtained.

Immediately after the peaks eluted from the columns, 4 ml was added to 75 ml of filtered lake water (FLW) and 4 ml to 75 ml of whole lake water (WLW), a 0.05-ml sample was plated for radioactivity and the balance retained in a sterilized test tube. One 25-ml aliquot of the fraction in FLW was passed through a 0.45-μ millipore and the anion exchange filter while another was passed through another 0.45-μ millipore and cation exchange filter assembly, Table 2. It is obvious that the high filter retention values for filtrate ^{32}P resulted from the colloidal ^{32}P. Perhaps an even more important result is that some of this fraction is negatively charged, as is the XP form.

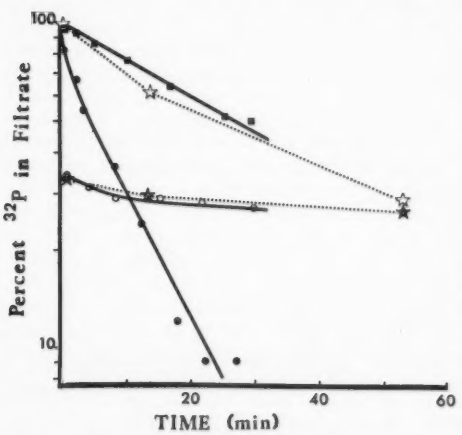

FIG. 6. Transfer curves of isolated filtrate fractions (○, colloidal ^{32}P; ■, $X^{32}P$; ●; $^{32}PO_4$ (peak 3 material) and shifts of colloidal ^{32}P (★) and $X^{32}P$ (☆) during delay experiments.

These experiments provide an explanation for Chamberlain's paradox. Even though colloidal ^{32}P and $X^{32}P$ are different from $^{32}PO_4$, they are anionic, to some degree, like $^{32}PO_4$. This means that they would perhaps act as $^{32}PO_4$ on ion exchange but their transfer rates are different from that for $^{32}PO_4$ — see below.

It is noteworthy to find that a small but significant amount of filtrate ^{32}P was retained by cationic ion exchange filters (Table 2). This amount ranged from 1 to 11% during the study. This retention was principally due to colloidal ^{32}P. By comparison, less than 1% of the $^{32}PO_4$ added to filtered lake water was retained by cationic ion exchange filters. This was unexpected and, at present, existing theory cannot explain the observation.

Transfer Rates of Filtrate Phosphorus Fractions

The "transfer" of radioisotope in each of the three principal filtrate peak materials by the particulate fraction was measured by adding 4 ml of each fraction to 75 ml of unfiltered lake water and millipore filtering as a function of time after addition (Fig. 6). The uptake of peak 3 ($^{32}PO_4$) material was similar to that for carrier free $^{32}PO_4$ and provides some evidence that there is no trace contamination to inhibit uptake and that peak 3 material is at least operationally the same as $^{32}PO_4$.

Transfer of the peak 1 (colloidal ^{32}P) fraction was 65% by the time the first data point was obtained. Although this extremely complicates any simple experimental design to measure transfer rates, it is not surprising since the retention on refiltration value was 46% (Table 2). Actual uptake was slow as shown by the slight slope in the line after the first data point. Peak 2 ($X^{32}P$) material was utilized at a log-linear rate, without filtration artifact, and with a rate constant less than that for $^{32}PO_4$.

The abiotic changes which took place were measured by adding 2.5 ml of the isolated fractions to 50 ml filtered lake water and, after 45 min, reinjecting samples into Sephadex G-25 gel filtration columns and noting any shifts in positions of the peaks. At the same time, samples of the isolated fractions were kept in sterile test tubes reinjected in G-25 columns as a control. The two control samples eluted at the same volume as before and little change occurred.

Peak 3 ($^{32}PO_4$) material remained unchanged (Fig. 7) $X^{32}P$ moved to the position of colloidal ^{32}P and that of $^{32}PO_4$. The colloidal ^{32}P sample was partly changed to $^{32}PO_4$.

From the August 11 data (Fig. 4, Table 1) for the abiotic changes in peak 1 and 2 material the following calculation was made. The 13.5% of the filtrate was

considered to be 100% XP and the fraction remaining at 15 and 55 min were calculated and plotted on Fig. 6 as well as the transfer data. Similarly, the 24.6% colloidal ^{32}P was plotted as 35%, (the first data point on the colloidal ^{32}P transfer) and the fraction remaining at 15 and 55 min were calculated. This shows that the transfer rate is equal to the rate at which both $X^{32}P$ and colloidal ^{32}P are converted to $^{32}PO_4$. These observations will be used in the development of a model consistent with the transfer kinetics but first the relative amounts of the several fractions of the compartments must be established.

As the asymptote level for the $^{32}PO_4$ transfer did not seem to change significantly between 2 and 48 hr, a typical 4.5-hr isotope distribution was used to estimate the relative amounts. At this time, 98.5% was in the seston fraction, 0.13% in the $X^{32}P$ fraction, and 1.16% in colloidal ^{32}P. Only 0.21% was in the peak material that corresponded to $^{32}PO_4$ (Fig. 8). As a typical value for total phosphorus in Heart Lake during this period is 44 μg/liter, (Rigler 1964), and if the nonlabelled phosphorus was distributed as the labelled phosphorus, then only 0.09 μg/liter was orthophosphate. This value is considerably below the sensitivity of the molybdate test for soluble reactive phosphate and approximates Rigler's (1966) estimate derived from a bioassay technique.

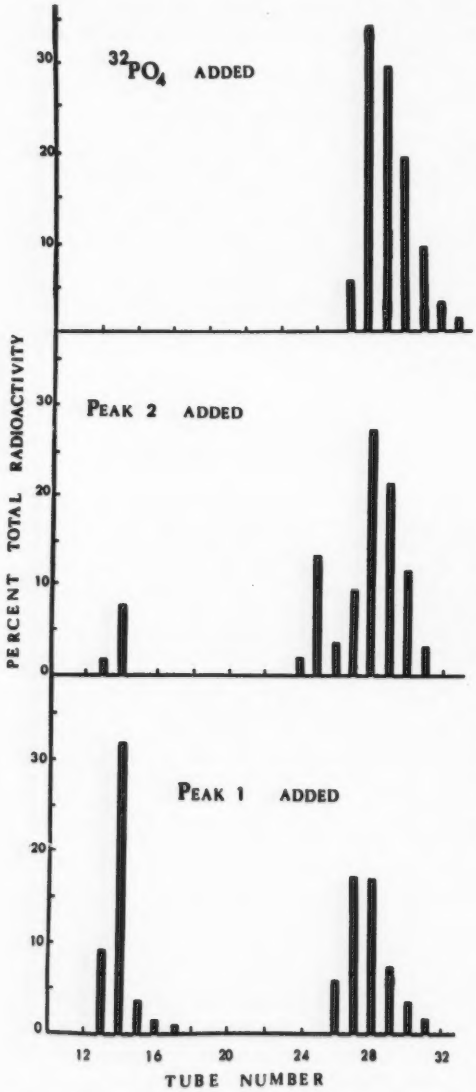

FIG. 7. Percentages of total radioactivities found in different phosphorus forms when three filtrate ^{32}P fractions were added to filtered lake water the abiotic changes were: $^{32}PO_4$ remained as $^{32}PO_4$, peak 2 material ($X^{32}P$) formed peak 1 material and inorganic ^{32}P, and peak 1 material (^{32}P) formed inorganic ^{32}P.

Discussion

The filtrate from whole lake water incubated with $^{32}PO_4$ contained two fractions in addition to $^{32}PO_4$. One fraction exceeded the upper limit for Sephadex G25 while the other had an apparent molecular weight of 250. The Sepharose 4B and Sucrose Density Ultracentrifugation of the large molecular weight fraction showed that this form of P exceeded 5,000,000 molecular weight (for polysaccharides), or 60S, and is referred to as colloidal-P although it may be in fact small particulate material. It seems possible that this form is similar to "particles" (Ogura 1970; Wagersky 1965; and Sheldon et al. 1967) detected in natural waters. The molecular weight 250 form was extremely labile and is simply referred to as XP. The four materials (seston, PO_4, colloidal P, and XP) were chosen in the development of a model consistent with the kinetics of isotope movement.

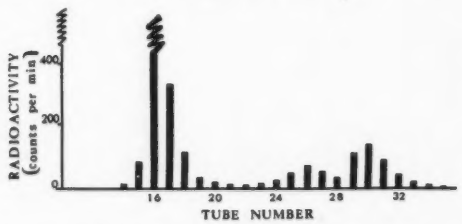

FIG. 8. Relative distribution of radioactivity in three filtrate forms (Sephadex G-25 separations) after lake water was incubated with $^{32}PO_4$ for 4.5 hr (max. 1430, jagged line).

The colloidal ^{32}P form was labelled within 2 min after $^{32}PO_4$ was added to whole lake water. Though direct complexing of $^{32}PO_4$ would seem to be a likely explanation, this hypothesis was rejected since $^{32}PO_4$ added to filtered lake water remained as $^{32}PO_4$. The possibility that it comprises organisms which are not retained by the millipore filter was also rejected since 0.1-μ filters did not completely remove the colloidal ^{32}P form. As direct complexing of $X^{32}P$ to colloids in filtered lake water was demonstrated, this would seem to be one explanation for the presence of colloidal ^{32}P.

The movement of radioisotope can be better understood by recalling the following observations:

(1) As colloidal ^{32}P and $X^{32}P$ were formed only when $^{32}PO_4$ was added to unfiltered lake water, their formation must involve "transfer" of $^{32}PO_4$ to the seston, with some tracer released quickly as $X^{32}P$. The direct complexing of $X^{32}P$ to unlabelled colloids then results in the formation of colloidal ^{32}P.

(2) Colloidal ^{32}P added to filtered lake water was partly hydrolysed to $^{32}PO_4$. No $X^{32}P$ was formed.

(3) When $^{32}PO_4$ was added to whole lake water and the seston removed by filtration, so that $X^{32}P$ and colloidal ^{32}P were both labelled, and when this filtrate was fractionated as a function of time after filtration, there was little change in the colloidal ^{32}P but the $X^{32}P$ form was converted to $^{32}PO_4$.

These observations indicate that the $X^{32}P$ molecules are excreted and quickly combine with colloids in the lake water. Subsequent additions of $X^{32}P$ displace the ^{32}P from the colloid making the $^{32}PO_4$ form available for uptake again. One explanation why the colloidal ^{32}P decreased in case (2) but not in case (3), is that the XP was labelled in (3) but not in (2).

A steady state at 4.5 hr (Fig. 8) was assumed and the isotope distribution used to represent the relative amounts of the four principal fractions (Fig. 9), and using data for the August 8–16 period the rate constants were either measured or calculated for these processes. This is similar to model previously introduced (Lean 1973a).

The initial slope of the transfer curve for $^{32}PO_4$ provides an approximation for the rate constant for uptake of $^{32}PO_4$, k_1 on the model (Fig. 1). It was found to be equal to 0.9 min^{-1}. The PO_4 fraction was 0.21% of the total isotope; therefore, the transfer of PO_4 must be $.0021 \times P_T \times .9 = 0.0019\ P_T$ μg/liter/min. (where P_T is the total biologically active phosphorus). I do not know P_T, since it is not measurable with existing technology, but since it is used in each calculation it can be omitted and the units of relative mass/min used.

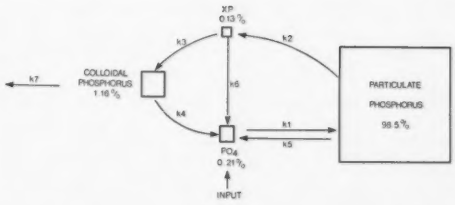

FIG. 9. A model for phosphorus movement within epilimnetic zone of lake waters, elucidating exchange mechanism between phosphate and the particulate fraction. A phosphorus compound (XP) is excreted and combines with a colloidal substance. This step involves release of phosphate from the colloid, making it available for uptake again. Some of the XP may be hydrolized directly to PO_4. A small amount of the colloidal P slowly becomes biologically unavailable and is lost from the system. This requires a fresh input of phosphate to maintain the system. Rate constants shown are: k_1 (uptake of PO_4) = 0.9 min^{-1}; k_2 (release of XP) and k_3 (binding of XP to colloidal P) = 0.022 min^{-1}; k_4 (hydrolysis of colloidal P to PO_4) = 0.0017; k_5 (release of PO_4 by seston), k_6 (direct hydrolysis of XP to PO_4), and k_7 (colloidal P becomes biologically unavailable)

One might argue that the initial fast transfer was due to removal of isotope by the filter itself. The experiments in which $^{32}PO_4$ was added to filtered lake water (Table 2) showed that, for periods up to 1 hr, no absorption or any other surface phenomenon involved $^{32}PO_4$. These experiments provide some justification for joining the first data point (20–40 sec) to 100%.

Colloidal ^{32}P and $X^{32}P$ were isolated using gel filtration and very small quantities were immediately added to samples of whole lake water and uptake curves obtained for these fractions (Fig. 6). The initial slope of the transfer curve provides the rate constants for transfer. These are 0.022 and 0.0017 min^{-1} for $X^{32}P$ and colloidal ^{32}P, respectively. These values are used in calculations below which show that considerable $^{32}PO_4$ is also released by the seston and that insignificant direct hydrolysis of $X^{32}P$ to $^{32}PO_4$, rather than via the colloidal form, takes place.

k_7 represents the rate constant for the process by which the colloidal ^{32}P becomes biologically unavailable. This is a slow rate relative to the movement of $X^{32}P$ to the colloidal and then to $^{32}PO_4$, and can be neglected in the following calculations. Nevertheless, this slow loss from the system must be balanced by fresh input of phosphate.

Doing a mass balance calculation on the colloidal ^{32}P fraction, k_4 times the relative amount of colloidal ^{32}P should equal k_3 times the relative amount for $X^{32}P$ plus direct hydrolysis to $^{32}PO_4$. In other

words, movement to the colloidal ^{32}P fractions is (.0013 × .022 relative mass units (rmu) min^{-1}), or .000029. The loss from the colloidal ^{32}P fractions is (.0017 × .0116 rmu min^{-1}) or .000020. These values are approximately the same showing that loss via the pathway shown as k_7 is much slower by comparison. PO_4 uptake (shown with rate constant k_1) was shown to be .0019 rmu min^{-1}. This is roughly 70 times greater than the production of $^{32}PO_4$ via the $X^{32}P$-colloidal ^{32}P pathway. This means that there is considerable direct release of orthophosphate from the sestonic pool (shown with rate constant k_5) or rapid cycling via the seston to XP followed by hydrolysis to PO_4; or that the 4.5-hr isotope distribution overestimated the PO_4 concentration by 70 times.

This overestimate could occur for two reasons. It has been demonstrated that $^{32}PO_4$ increased in the filtrate with time after filtration. Even though the sample was injected immediately there was a delay before the filtrate fractions were sufficiently separated for this reaction to stop. Secondly, the $^{32}PO_4$ pool might decrease further over the next few days. To study this possibility, lake experiments rather than experiments on isolated samples of lake water must be conducted.

Transfer by the seston must equal release but since I was unable to measure the rate constants shown as k_2, k_5, and k_6, the others cannot be calculated. Consequently, the relative importance of release of phosphate or XP cannot be determined. However, since the transfer of XP was approximately the same as that of colloidal P (Fig. 6), the direct hydrolysis of XP to PO_4 appears to be insignificant.

The model (Fig. 9) can be used to explain the transfer kinetics observed when $^{32}PO_4$ is added to unfiltered lake water. The initial rapid transfer occurs as $^{32}PO_4$ is removed by the filtrable fraction. Departure from log linearity occurs when significant amounts of $^{32}PO_4$ and $X^{32}P$ are released by the seston. As the $^{32}PO_4$ becomes low, the rate at which $X^{32}P$ combines with colloidal ^{32}P, liberating $^{32}PO_4$, becomes significant and this results in the deviation from the kinetics consistent with a two-compartment exchange model. The distribution of isotope then assumes the steady state, with most of the total isotope in the seston but most filtrate ^{32}P in the colloidal form.

Some improvements must be made in the model when we consider the changes in isotope distribution during a transfer experiment. If the seston was only one homogeneous material, the maximum amount of isotope in the XP fraction would be found when the amount of ^{32}P in the seston was greatest. Instead, $X^{32}P$ was maximum 15 min after $^{32}PO_4$ was added to the whole lake water (Table 1). The XP must therefore be formed from recently obtained $^{32}PO_4$. This means that the seston must be subdivided into at least two fractions: one that is involved with rapid exchange with the solution forms and another that is not. This observation is consistent with Rigler's (1972) washout experiments. He found that sestonic P was initially released rapidly followed by a further loss at a much slower rate.

The observations which led to the model also explain some formerly puzzling results from ion exchange experiments. It had been assumed that nonorthophosphate forms of dissolved phosphorus would not be anionic, but it is now obvious that some of the colloidal ^{32}P and all of the $X^{32}P$ is anionic (Table 2). This explains the paradox in Chamberlain's (1968) work: filtrate ^{32}P eluted like $^{32}PO_4$ on Dowex X8 ion exchange columns but was utilized at a very different rate. Even though these forms are anionic, the rates of movement to the filtrable fraction differ from that for $^{32}PO_4$.

The shape of the filtrate ^{32}P transfer curves can now be explained more fully. The "instantaneous uptake" was due to retention of colloidal ^{32}P. Further transfer by seston involves transfer of the small amounts of $^{32}PO_4$ and $X^{32}P$, and the available part of the colloidal ^{32}P. The high asymptote results from the portion of the colloidal ^{32}P which is biologically unavailable.

The model predicts that the terminal ^{32}P on the colloidal is replaced on $X^{32}P$ additions. Both forms are negatively charged but, if a positive ion (Ca^{++}, Fe^{+++}, etc.) binds to this negative site, its ion exchange properties would be destroyed. This may explain why some colloidal ^{32}P is biologically unavailable. Further binding of XP would also be prevented.

These observations on the ion exchange properties of colloidal ^{32}P are relevant to Rigler's 1968 studies using hydrous zirconium oxide (HZO) columns to separate orthophosphate from the hydrolysable phosphorus fraction and soluble unreactive phosphorus. He postulated that large organic molecules were present that did not penetrate the pores of the ion exchange material and the ion exchange sites on the surface quickly became saturated; however, $^{32}PO_4$ continued to be removed at a high efficiency. My observations support Rigler's postulate. Anionic colloidal ^{32}P was removed at a high efficiency when small volumes were passed through the columns. As the molecular weight exceeds 5,000,000, it seems probable that saturation of the ion exchange sites on the surface would occur.

The filtrate ^{32}P delay experiments which showed a shift from $X^{32}P$ and colloidal ^{32}P to $^{32}PO_4$ offer an alternative explanation for King's (1970) conclusion that cells were sufficiently damaged during filtration to release some cellular phosphate. Using a bioassay measurement for phosphate concentration, he reported that the amount of phosphate released by cells during filtration was approximately the same as the amount of orthophosphate present. Most of the filtrates King used had been filtered for some time before his experiments were started. If the distribution of isotope in my experiments represents the distribution of phosphorus compounds, the delay between time of filtration and his experiments could account for at least a twofold increase in phosphate concentration.

Our present knowledge of phosphorus dynamics is insufficient to relate the model to existing literature. Rigler (1972), states that there are three possible mechanisms for returning PO_4–P to solution: (1) direct release of P by the ultraplankton; (2) excretion of P by zooplankton; (3) enzymatic hydrolysis of organic P compounds excreted by organisms or produced by autolysis or decomposition of dead plankton.

He points out that the most important source of phosphate regeneration has been stressed by different workers but no systematic analysis of the relative importance of either mechanism is possible. He calculated that excretion by zooplankton and direct release from ultraplankton were equally important mechanisms.

In my experiments, the removal of zooplankton did not affect the transfer kinetics done immediately after filtration. This does not exclude the possibility that I measured small pools with fast turnover times. The relative importance of these pathways requires simultaneous measurements of them. This has not been done and, at the present time, this model cannot be used in the context of total lake metabolism.

I have shown that a two-compartment exchange model such as that proposed by Rigler (1956) must be modified to include significant excretion of an XP form which combines with colloids in lake water. This step involves the liberation of phosphate from the colloid making it available for transfer again. Most of the phosphorus in solution is in the colloidal ^{32}P form and some of it becomes biologically unavailable.

The seston releases not only an element considered to be the limiting nutrient but also an organic phosphorus compound. This quixotic behavior appears to be unprofitable for the organisms involved and the reason remains obscure. Perhaps it is a stress response to high biomass and/or low phosphate concentration.

Extension of Model Shield Lakes

During the summers of 1971 and 1972, experiments were conducted on lakes 227, 304, 239, 302, and 304 of the Experimental Lakes Area of the Freshwater Institute, Fisheries Research Board of Canada, Winnipeg. These lakes have been previously described (J. Fish. Res. Board 28(2), 1971). Although these lakes differ from each other, from Heart Lake, and in some cases from those earlier reported by Rigler (1956), all of the transfer curves could be fitted using only a single exponential even though all contained the same three forms of phosphorus as Heart Lake. After 1 hr, most of the $^{32}PO_4$ was contained in the colloidal ^{32}P form (Lean and Schindler unpublished data). Presumably, the rate at which XP and colloidal P became labelled was sufficiently slow that it did not cause the kinetics to deviate from a two-compartment exchange model.

These studies indicate that the observations made on Heart Lake, a hard water eutrophic lake also apply to a continuum of lakes varying from oligotrophy to eutrophy on the Canadian Shield.

Acknowledgments

I am indebted to Dr F. H. Rigler, Department of Zoology, University of Toronto, for his advice and encouragement during the course of this investigation. The research was funded by grants to Dr F. H. Rigler from the Fisheries Research Board of Canada and the National Rescarch Council of Canada. I would also like to thank Dr D. W. Schindler, Freshwater Institute, Fisheries Research Board of Canada, Winnipeg, for his criticism of the manuscript.

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Measurement of Adenosine Triphosphate (ATP) in Two Precambrian Shield Lakes of Northwestern Ontario

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RUDD, J. W. M., AND R. D. HAMILTON. 1973. Measurement of adenosine triphosphate (ATP) in two Precambrian Shield lakes of northwestern Ontario. J. Fish. Res. Board Can. 30: 1537–1546.

Profiles of the adenosine triphosphate (ATP) concentrations occurring in the particulate material of two experimental shield lakes were obtained with respect to time. Large seasonal changes were evident, as were specific sites of high biological activity due either to natural stratification or to deliberate manipulation. Chlorophyll *a*, particulate carbon, and direct count data were found to be misleading in specifying the changes or the sites of biological activity. Analysis of the ATP data on the basis of the relative abundance of different size fractions was found to be useful. An ATP filtration error could not be demonstrated, indicating that cell breakage is not a source of the filtration error inherent in some ^{14}C primary and secondary productivity studies.

RUDD, J. W. M., AND R. D. HAMILTON. 1973. Measurement of adenosine triphosphate (ATP) in two Precambrian Shield lakes of northwestern Ontario. J. Fish. Res. Board Can. 30: 1537–1546.

Les auteurs ont obtenu des profiles de concentration d'adénosine-triphosphate (ATP), en fonction du temps, dans le matériel particulaire de deux lacs expérimentaux du Bouclier. Ces profiles révèlent de fortes variations saisonnières et identifient les sites spécifiques de forte activité biologique causée soit par une stratification naturelle soit par une manipulation volontaire. La chlorophylle *a*, le C particulaire et les comptages directs sont trompeurs comme indicateurs de ces variations et des sites d'activité biologique. Par ailleurs, l'analyse des données de l'ATP sur une base d'abondance relative de fractions de différentes grandeurs s'avère utile. L'absence d'erreur de filtration d'ATP indique que la rupture cellulaire n'est pas une source d'erreur de filtration inhérente à certaines études de productivité primaire et secondaire au ^{14}C.

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THE assay of adenosine triphosphate (ATP) in ocean water samples was designed to assist in the solution of a classical microbiological problem; that of assaying total living heterotrophic biomass. ATP is characteristic of all living cells (Huennekens and Whitely 1960), is lost very rapidly upon cell death (Holm-Hansen and Booth 1966), is not associated with inert particulate material (Holm-Hansen and Booth 1966; Hamilton and Holm-Hansen 1967), and by reason of the great depths at which samples are taken in the ocean can be reasonably presumed to be associated with non-autotrophic organisms. This latter factor precludes the exclusive assay of heterotrophs in areas suspected to contain autotrophs, i.e. lake waters.

Analysis of ATP in deep ocean samples requires either the filtration of prohibitively large samples or the purchase and use of very sensitive instrumentation. This factor, plus a preoccupation with the exclusive assay of nonautotrophs, seems to have inhibited the application of the ATP assay to lakes.

Printed in Canada (J2823)

Materials and Methods

Samples for ATP analysis were obtained from lakes 227 and 302 of the Experimental Lakes Area, northwestern Ontario. Lake 227 has been artificially eutrophied by additions of 0.34 g P and 5.04 g N/m^2 per year commencing in 1969 and 0.48 g P plus 6.3 g N/m^2 each year since (Schindler et al. 1971, 1973). The hypolimnion of the north basin of lake 302 received additions of 0.26 kg P, as H_3PO_4, 1.39 kg N as NH_4Cl, and 1.85 kg C as sucrose/ha per week during the period of June 8, 1972 – September 13, 1972 (D. W. Schindler unpublished data).

SAMPLE COLLECTION AND EXTRACTION

Lake water samples, collected in a Van Dorn bottle, were transferred to polyethylene sample bottles which had been previously rinsed with sample water. Between samplings the sample bottles were washed twice with

distilled water and allowed to air dry. Sterilization was found to be unnecessary. Samples were maintained at 4 C and extracted within 3 hr of sampling. However, euphotic zone samples have been stored for up to 14 hr in opaque bottles at room temperature without significant change in ATP activity (E. J. Fee personal communication).

Before extraction each sample was passed through a 250-μ nylon net to remove the larger zooplankton. Three subsamples of appropriate volumes were prepared. One was passed through 0.22-μ 47-mm GS Millipore® filters. The other two were passed through 10- or 56-μ nylon mesh filters cut to fit the base of the Millipore filtering apparatus. In this case a stainless steel support screen was used, as a normal sintered glass base tended to clog. After sample extraction the nylon mesh filters were washed for 30 min in boiling distilled water.

A vacuum of 80 mm Hg was used during filtration in an attempt to minimize the danger of cell breakage on the filters.

ATP extraction was similar to the procedure of Holm-Hansen and Booth (1966) except that autoclaved 0.05M Tris buffer (*p*H 7.7) was used instead of 0.02M Tris buffer (*p*H 7.75).

ATP Analysis

Luciferin-luciferase preparation — Lyophilized firefly lantern extracts were obtained from Sigma Chemical Co. The enzyme was stored at –20 C until 3 hr before use. At that time it was rehydrated with 6 ml of autoclaved Tris buffer. The suspension was shaken vigorously for approximately 30 sec after rehydration. It was then left for an hour at room temperature in the light before it was reshaken for a further 30 sec. Particulate matter in the vial was then allowed to settle and the clear supernatant was used in the analysis.

Equipment — The analyses were performed under diffuse incandescent light in modified glass scintillation vials similar to those described by Schram (1970). These vials permitted the use of small volumes, yet maintained the geometry of the counting system. The scintillation vials were constructed by glueing plastic caps from disposable centrifuge tubes into the center well of the underside of the lids of glass scintillation vials. These caps fitted tightly in $\frac{1}{4}$-dr 9 × 30-mm shell vials. The assembled apparatus is depicted in Fig. 1.

The centrifuge tubes themselves should not be used due to chemi-luminescence induced by exposure to light.

The ATP analyses were carried out either on a Model 3310 Packard Tri-Carb scintillation counter or a Picker-Nuclear Liquimat 220 scintillation counter. As suggested by Stanley and Williams (1969) both counters were operated out-of-coincidence. Operated in this manner the thermal noise counts of both machines were approximately 200 counts/0.1 min at the desired window settings. This count rate is insignificant in comparison to the counts obtained during ATP analyses. In the case of the Packard Tri-Carb counter, the coincidence circuit is switched out. With respect to the Picker-Nuclear counter either of the photomultiplier tubes can be deactivated. In practice, the phototube with the least thermal noise was chosen.

The first time a scintillation counter was used for ATP analysis window settings were chosen from an ATP pulse height spectrum which was obtained by the channels ratio method of Stanley and Williams (1969). These window settings (25–40 for the Picker-Nuclear and 30–50 for the Packard Tricarb) varied slightly from day to day, according to the activity of luciferin–luciferase. Therefore, a precise setting was conveniently obtained by adjusting the window size to give an enzyme blank count of 20,000 counts/0.1 min (an enzyme blank consisted of 0.1 ml of the enzyme preparation plus 0.2 ml of autoclaved Tris buffer). From experience it had been noted that a window setting which gave this blank count would result in a standard curve in which the highest ATP concentration (4×10^{-3} μg/0.2 ml) would not saturate the scalers but for which the lowest concentration (2×10^{-4} μg/0.2 ml) was still about 15,000 counts above the enzyme blank value.

Standard curve — A standard ATP solution consisted of 11.93 mg ATP-$Na_2 \cdot 3H_2O$ (Sigma Chemical Co.) in 1.0 liter of autoclaved Tris buffer. This solution was stored at –20 C in 5-ml quantities. It was stable for months at this temperature. Immediately before analysis 1.0 ml of the solution was serially diluted to yield ATP concentrations of 4×10^{-3}, 2×10^{-3}, 4×10^{-4}, and 2×10^{-4} μg ATP/0.2 ml.

A standard ATP curve was constructed as follows: One-tenth milliliter of enzyme extract was pipetted into a shell vial. This was followed by 0.2 ml of a standard ATP solution. The modified scintillation vial was then assembled as described previously. The assembly usually required approximately 5 sec. The modified vial was then shaken rapidly until 20 sec had elapsed at which

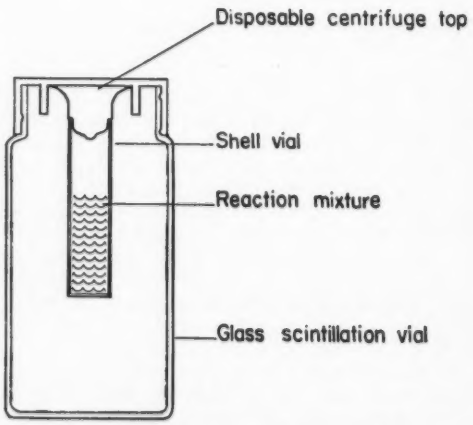

FIG. 1. Cross section of a modified glass scintillation vial containing the reaction mixture in the inner shell vial.

time it was placed into the scintillation counter. The 0.1-min count began at exactly 30 sec.

The activation of the two scintillation counters was somewhat different. In the case of the Packard Tri-Carb, which was used in the automatic mode, the scintillation vial was placed onto the sample changer and the sample changer switch was set to repeat at exactly 20 sec. The sample would then begin to count at exactly 30 sec since there is an inherent 10-sec delay when the Packard is operated in this manner.

The Picker-Nuclear counter was operated in the manual mode. The modified vial was lowered into position at 20 sec by activating the down button. At exactly 30 sec the start button was activated to begin the 0.1-min count.

An enzyme blank consisting of 0.1 ml enzyme and 0.2 ml of autoclaved Tris buffer (0.05 m *p*H 7.7) was run in exactly the same manner as the four standards. The enzyme blank count was then subtracted from the four ATP standard counts to obtain net counts used in the preparation of the standard curve (Fig. 2).

If this procedure was followed rigorously, duplicate ATP standards agreed to within less than 1% at higher concentrations and to within less than 5% at the lowest concentration of the standard curve.

Sample analysis — Ten minutes before analysis frozen ATP samples were thawed in a water bath adjusted to room temperature (the same temperature as the enzyme preparation).

Analysis of samples was carried out in exactly the same manner as for the ATP standards. This procedure required less than 1 min/sample.

After every five samples an enzyme blank was run to account for the progressive decrease of the endogenous activity of the luciferin–luciferase. The enzyme blank value was then used to calculate net counts for the previous five ATP samples. The net ATP counts were then used in conjunction with the standard curve to read off the concentration of ATP in 0.2 ml of sample. The concentration of ATP in 0.2 ml of sample was related back to lake water concentration with the following equation:

$$\mu g \text{ ATP/liter lake water} = \frac{\mu g \text{ ATP}/0.2 \text{ ml sample} \times 5 \times \text{vol. of extract } (ml) \times 10^3}{\text{ml of lake water filtered}}$$

Using this method the standard error of six extracts of the same lake water sample was normally about 6%.

An estimate of live carbon concentration can be obtained by multiplying ATP concentration by 250 (Holm-Hansen 1969).

Particulate carbon analyses were carried out as described in Armstrong and Schindler (1971). Chlorophyll *a* (uncorrected for phaeophytin or phaeophorbides) was determined by the fluorometric procedure of Yentsch and Menzel (1963). Oxygen was determined by the azide modification of the Winkler method in Standard Methods (American Public Health Association 1965).

Results

Two lake 227 ATP profiles are presented in Fig. 3. These data represent the ATP occurring in the 0.22–250-μ size fraction. Thus, with the exception of organisms greater than 250 μ, these curves may be taken to represent the "total" microbial biomass of the lake. The two curves show the highest and

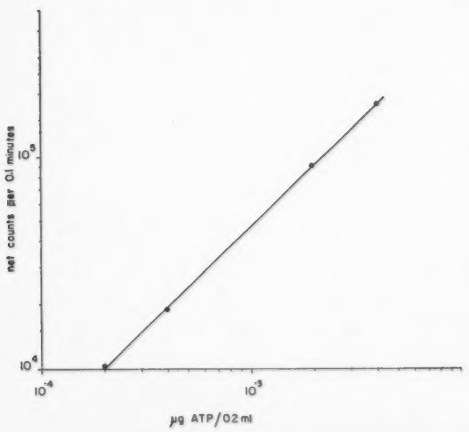

FIG. 2. Plot of ATP concentration against net counts/0.1 min; net counts were calculated by subtracting enzyme blank counts from counts obtained from standard ATP concentrations.

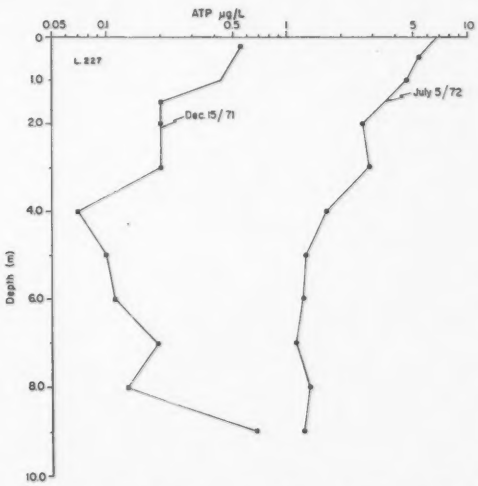

FIG. 3. Vertical distribution of the 0.22–250-μ ATP size fraction in lake 227 showing the highest and lowest seasonal concentrations.

the lowest ATP concentrations found in lake 227 during the year. The large fluctuations in living material, known to occur in lakes seasonally, are clearly evident. Moreover, the profiles indicate complex seasonal changes in the distribution of living material with depth.

These changes are more evident when a series of these ATP profiles are plotted as an isopleth (Fig. 4). For instance, the first 3 m of lake 227, which during the ice-free period usually comprise the euphotic zone of the lake, show seasonal fluctuations in ATP concentration reflecting the size of the autotrophic and heterotrophic microbial populations. ATP concentration quickly receded between 1 and 3 m during September and October 1971. This decrease continued at a slower pace until December 1971.

During the remainder of the winter there was a slight increase in ATP concentration beneath the ice. After ice-off ATP levels in the euphotic zone approached the high value of the previous summer as the phytoplankton population increased. However, this intense algal bloom reached a maximum at the end of July 1972, then suddenly died off, resulting in reduced euphotic zone ATP concentration early in August.

Between 3 and 7 m of lake 227 there was a zone of lower ATP concentration which also exhibited seasonal fluctuations. Below 7 m there was usually a second peak in ATP concentration which increased towards the sediments. The origin of this bottom water ATP peak is discussed later.

Figure 5 is presented in order to illustrate that the amount of information generated by the method can be increased by considering the ATP associated with the particulate material retained by various sizes of filters. It should be noted that all reported concentrations represent material passing a 250-μ filter but retained by the stated size. Thus the 10-μ data refer to the ATP in material 10–250 μ in size, not 10–56 μ in size.

Other sizes of filters were used during initial experimentation (0.1-, 0.22-, 0.45-, 1-, 2-, 5-, 8-μ pore-size membrane filters and 10-, 40-, 56-, 73-, 100-, 250-, and 500-μ mesh netting). In practice, it was found that the 0.22-μ filter and the 10- and 56-μ nets yielded the most significant size separations and that usually the other sizes were redundant. This selection of sizes can be expected to vary from lake to lake and should be checked before routine sampling is attempted. Analysis of material retained on net sizes >250 μ cannot be recommended nor can prefiltration using larger nets, because one large zooplankter can contain many times the ATP associated with the sample sizes used to obtain microbial biomass data. Moreover, our water sampling method cannot produce a sample volume large enough to yield statistically meaningful data regarding the distribution of larger forms and can produce significant avoidance reactions in these animals. Given a proper sampling method, an extraction technique which could be used in the field and which is effective with large

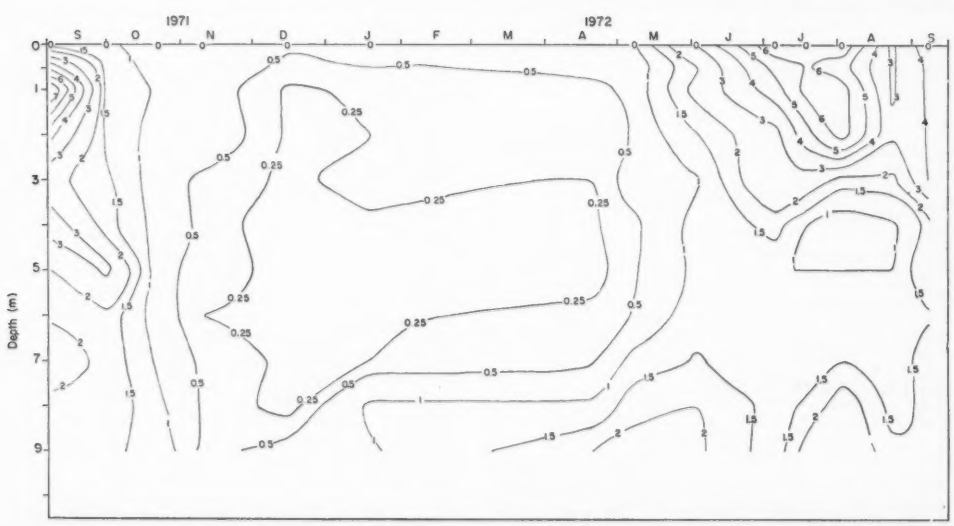

FIG. 4. Isopleth of the 0.22–250 μ ATP size fraction (μg/liter) of lake 227 during 1971–72.

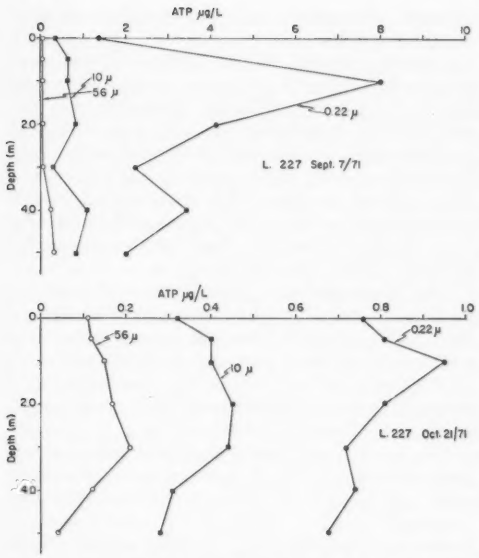

FIG. 5. Vertical distribution of three ATP size fractions in lake 227, 0.22–250 µ (closed circles), 10–250 µ (squares), and 56–250 µ (open circles).

animals, it would appear that the method could be extended to the assay of plankton >250 µ.

In considering the "total" microbial biomass represented by the 0.22-µ lake 227 data of Fig. 5, one again sees temporal changes in ATP concentration as well as changes in the distribution of ATP with depth. More importantly, however, the various size fractions illustrate a fundamental change in lake 227: the transition from the dominance of summer phytoplankton to the dominance of typical winter phytoplankton.

In late summer (September 7, 1971) 5% of the biomass between the surface and 3 m was found to be greater than 10 µ while only 1% was greater than 56 µ. However, after winter populations appeared (October 21, 1971), approximately 50% of the biomass between the surface and 3 m was greater than 10 µ, whereas 19% was greater than 56 µ. This observation was found to be consistent with direct count phytoplankton data. Chlorophyta of <10 µ predominated in summer phytoplankton while biomass contributed by Cryptomonads and Chrysophyceae which were >10 µ was relatively more important in winter populations (H. Kling personal communication). The percentages of ATP biomass greater than and less than 10 µ in summer and winter populations between the surface and 3 m, is compared to biomass calculated from direct phytoplankton counts in Table 1. These data demonstrate that both ATP and direct count data detected increases in the percentage of biomass >10 µ with the appearance of winter phytoplankton populations. The percentage values differ, however, and this may reflect the value judgements of viability that are required during direct phytoplankton counting biomass estimation and of course the inclusion of ATP from nonphytoplankton sources.

After freeze-up on lake 227 (November 4, 1971) the shape of the October 21 curve shown in Fig. 5 began to assume the more typical winter shape shown in the December 15 curve of Fig. 3. That is to say there is a pronounced maximum immediately underneath the ice. This maximum never reached more than 10% of the peak values recorded from the epilimnion in the summer.

Many limnological studies generate data regarding the distribution of particulate carbon and chlorophyll *a*. It has long been suspected that such data do not indicate sites of biological activity and this suspicion can be easily confirmed through the use of the ATP assay. In Fig. 6 profiles of the two classical parameters are compared with an ATP profile using a typical set of data. On this date the chlorophyll *a* data (uncorrected for pheophytin and phaeophorbides) indicated a peak near 7 m while the ATP data indicated that this peak was largely composed of dead material. At 3 m there was a peak in particulate carbon which occurred at a minimum in ATP concentration. The ATP maximum at 1 m was reflected in neither the particulate carbon nor the chlorophyll *a* data.

The usefulness of ATP data in pinpointing sites of high biological activity is best illustrated through a profile from the north basin of lake 302. The north basin of this lake was receiving weekly fertilizations delivered into the hypolimnion at a depth of 8 m. The samples which provided the data shown in Fig. 7 were taken 2 m away from the fertilization pipe about 2 months after fertilization commenced.

TABLE 1. A comparison of the percentage of biomass between 0 and 3 m of lake 227, as calculated for ATP data and direct count phytoplankton data, which were greater than or less than 10 µ before and after the appearance of winter phytoplankton populations.

	Summer population Sept. 7, 1971		Winter population Oct. 21, 1971	
	>10 µ	<10 µ	>10 µ	<10 µ
ATP biomass	5%	95%	50%	50%
Direct count biomass	20%	80%	66%	33%

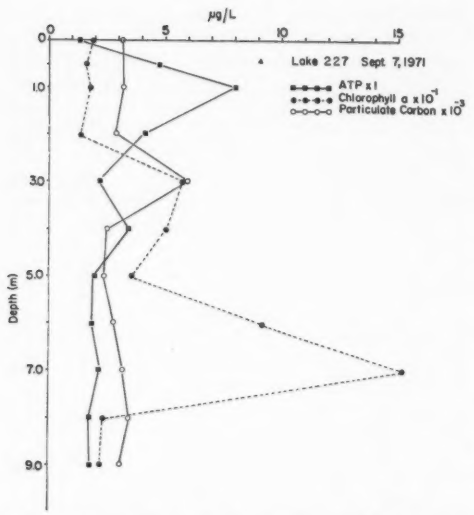

FIG. 6. A comparison of an ATP profile (squares) with chlorophyll *a* (broken line) and particulate carbon profiles (open circles). On this date the thermocline extended from 4 to 6 m.

The highest ATP concentrations in the north basin were found below 8 m with a maximum at 9 m. In contrast, in the south basin, which was unfertilized (Fig. 7), the ATP profiles of the three size fractions decreased toward the sediments and the 9-m values were the lowest recorded in the profiles.

The organisms producing the large ATP peak in the hypolimnion of the north basin were virtually all less than 10 µ since the peak appeared on 0.22-µ membrane filters but not on the 10- and 56-µ nylon mesh filters. These organisms must have been mostly bacteria and/or yeasts since direct counts showed no significant phytoplankton population (H. Kling personal communication).

Oxygen depletion was evident in the region of the north basin ATP peak (Fig. 7). The boundary of the oxygenated and deoxygenated water (8–9 m) also was the depth at which the ATP size fractions changed abruptly. At 8 m, 70% of the ATP was found to be in particles larger than 56 µ, while at 9 m and at a reduced oxygen concentration only 5% of the ATP was larger than 56 µ. The boundary appears to be the point at which a small aerobic biomass was supplanted by a large fermentative heterotrophic microbial population. The relatively large contribution to the total ATP assays made by the 10- and 56-µ fractions of the samples for 8.0 m and above may be due to the occurrence of a copepod *Diaptomus* sp. (M. Buckley personal communication).

Another example of the ability of this assay to specify a site of biological activity has been obtained from lake 227. The December 15, 1971 curve in Fig. 3 indicates an ATP peak very close to the bottom of the lake. The increase of this peak in relation to the total water column is shown in Fig. 4. It can be seen that it continued to grow in size and concentration until June 2 of the next year; its persistence may have been due to incomplete turnover during the spring.

During this periodA TP profiles were also prepared using 10- and 56-µ filters. These profiles indicated that >90% of the ATP of the bottom water peak existed in particles of <10 µ, since <10% and <1% of the total ATP was retained by the 10- and 56-µ filters, respectively. Thus the ATP peak was largely composed of bacteria-sized particles.

On two occasions during this period direct counts were carried out on a 9-m water sample to determine phytoplankton biomass. ATP values were compared to these estimates by converting both estimates to carbon (ATP × 250; assume phytoplankton cells to contain 5% C/unit volume). In both instances this biomass could not nearly account for the observed ATP measurements (H. Kling personal communication). A population of zooplankton of <10 µ is also unlikely to account for this ATP peak since the peak occurred in anaerobic water. Lake 227 profiles from 5 to 9 m during the period of November 9, 1971–July 5, 1972 of living particulate carbon (as derived from the ATP analyses) are shown in Fig. 8. Also plotted in this figure are particulate carbon data for the same time interval. It is evident from Fig. 8 that the majority of the particulate carbon was dead throughout this time interval. However, there appears to be a relation between changes in ATP particulate carbon concentrations and particulate carbon concentrations. For instance, at 9 m the highest particulate carbon concentration occurred on April 19, 1972. This was followed by a maximum concentration of ATP at 9 m on May 7, 1972. After April 19, 1972 the particulate carbon at 9 m consistently decreased. This was also reflected in reduced 9-m ATP particulate carbon concentrations after May 7, 1972. Similar trends can also be seen at other depths with increases in ATP particulate carbon estimations usually lagging behind increases of particulate carbon concentrations.

Discussion

Although the only adjustment to standard extraction procedures that we found necessary was a change in sample volume, one should be aware

that Lee et al. (1971) have reported difficulties in applying the ATP assay to lake sediments. Therefore lakes receiving a high burden of sediment should be assayed with caution. Particulate material was not a problem in lake 227 because a fiftyfold increase in particulate concentration in 0.22-μ ATP extracts has no effect on the ATP analysis.

Some primary production studies employ a filtration step and it now is recognized that, under certain circumstances, this can introduce error (Arthur and Rigler 1967). Similar observations have been made with respect to the assessment of the activities of heterotrophic microorganisms (Thompson and Hamilton 1973). In the case of primary production studies the "filtration error" has been tentatively attributed to cell breakage during filtration and if true, would certainly affect ATP analyses. Consequently the possibility was investigated by performing standard filtration correction curves (Arthur and Rigler 1967) on lake samples and by repeating the procedure using axenic cultures of a ciliate protozoan (*Tetrahymena* sp.) which, from personal experience, breaks under hard filtration. Under the conditions we employed during filtration, a filtration correction curve for ATP could not be demonstrated using either natural samples or the delicate protozoan. We therefore conclude that with careful filtration cell breakage is an unlikely cause for the "filtration error" found during ^{14}C experiments and that the error is not pertinent to ATP assays. These data confirm the claim of Nalewajko and Lean (1972) that the "Arthur–Rigler" correction was not a result of cell breakage on the membrane but was likely caused by retention of the ^{14}C label by membrane filters.

Procedural details appear secondary to the application of this assay to lake waters and the basic question is the utility of applying the ATP assay in lakes.

Athough the use of the assay in lakes is not restricted to estimating heterotrophic organisms, it can be used to discriminate between areas of high and low biological activity. For example, our ATP profiles usually show a peak near the surface; a

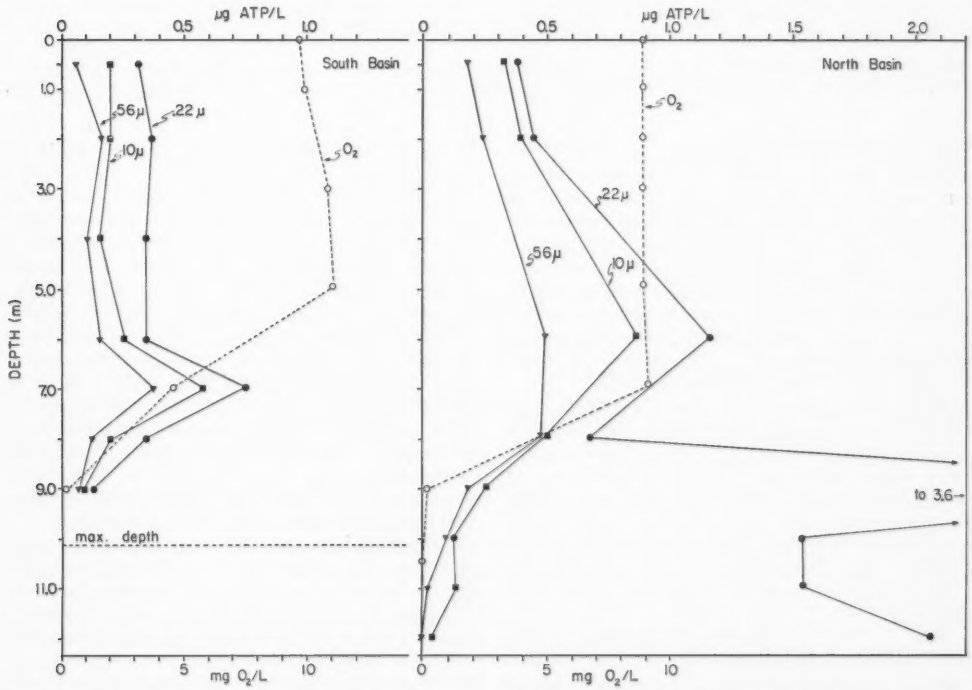

FIG. 7. Vertical distribution of three ATP size fractions in the north and south basins of lake 302; 0.22–250 μ (closed circles), 10–250 μ (squares), and 56–250 μ (triangles). The north basin was receiving a weekly fertilization delivered into the hypolimnion at a depth of 8 m.

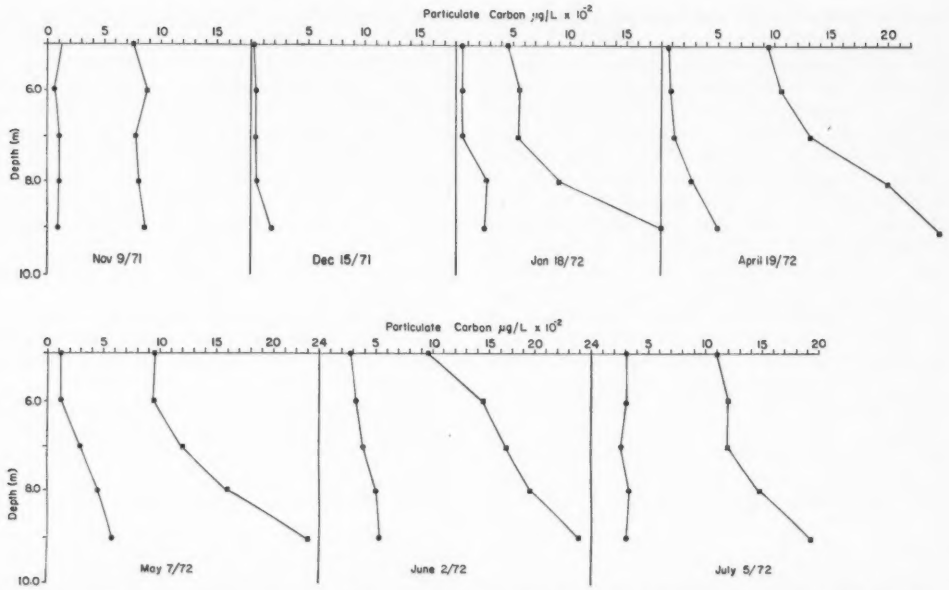

FIG. 8. Comparison of particulate carbon concentrations (squares) and ATP concentrations which have been converted to live carbon (closed circles) in lake 227. Particulate carbon data was unavailable for December 15, 1971.

peak which would be expected from a usual distribution of phytoplankton. However, through the use of ATP assay one can quickly delineate the precise layer containing the highest concentration of living phytoplankton. Data such as these permit decisions to be reached regarding the relative effort which must be expended upon sampling certain depths and could assist one in reaching a decision as to the priority assigned to samples being assayed by such time-consuming procedures as direct phytoplankton or zooplankton counting.

A second example of the ability to delineate sites of biological activity is presented in the data from the north basin of lake 302 which is being enriched with carbon, nitrogen, and phosphorus below the thermocline (Fig. 7). In this instance it was possible to demonstrate the existence of a persistent lens of very high ATP occurring at about 9 m. This picture can be compared with the normal profiles from the comparable, but unenriched, southern basin of the lake (Fig. 7).

A third example is contained in the data for lake 227 in which a peak of high ATP concentration was detected near the bottom and whose development was followed throughout the year (Fig. 4).

The existence the peaks of activity mentioned in the last three examples would be quite unsubstantiated on the basis of data normally collected during a limnological study.

Rather more detailed information regarding the size distribution of living material is obtained from the method if the particulate material is separated on the basis of size prior to analysis. In lake 227 it has been shown that the change in the relative contribution of certain size classes to the pattern of the ATP profile accurately predicted the change from typically summer to typically winter phytoplankton populations. In addition, the ATP peak near the bottom of this lake can be attributed to microorganisms $<10\ \mu$. In lake 302 it has also been shown that microorganisms $<10\ \mu$ are primarily responsible for the peak of activity at 9 m. However, a peak around 6 m was also detected which was associated with a different size fraction.

Typically, phytoplankton and zooplankton analyses are not available to the limnologist until long after most of the chemical data have been received. The same observations can be made of most chemical analyses devoted to the assay of particulate material. The ATP assay thus provides an alternate method which can detect quickly and easily changes in the amount of distribution of living material. The ATP data can be generated as easily as chlorophyll data, if one has a scintillation counter, and with

certain refinements to the method there appears to be no reason why ATP profiles could not be produced quickly if the investigation requires real time data.

The lack of correlation between the ATP, chlorophyll *a*, and particulate carbon data is not surprising since the three procedures assess quite different parameters. When phytoplankton sedimented out of the euphotic zone of lake 227 (which was usually about 3 m in depth) they died quickly according to ATP data. The deep chlorophyll *a* peaks and the associated particulate carbon peaks were usually found to be mostly composed of dead material. When one is considering cycling of nutrients within or through a lake, analyses such as particulate carbon would be useful. However, if particulate carbon and chlorophyll *a* data were interpreted in terms of physiological activity they would often be misleading. ATP measurement seems to be the only reliable means of measuring the location and magnitude of living biomass in lakes.

While our intent was to demonstrate only the utility of the approach it appears possible to make some comments on two of the lakes sampled during this investigation.

During the winter months there was increasing biological activity in the bottom waters of lake 227. The growth of this bottom water ATP peak was believed to be linked to an increase in particulate carbon, which probably sedimented from higher in the water column during the winter months. The bottom water ATP peak was believed to be largely composed of bacteria and yeasts since it occurred largely in anaerobic water, the particle size was <10 μ, and direct counts showed no significant phytoplankton population. The highest recorded single ATP concentration of the bottom water peak was 2.3 μg ATP/L on May 7, 1972. This value was 33% of the highest ATP concentrations that occurred in the epilimnion during the next summer, demonstrating the importance of this fermentative biological activity in the metabolism of the lake as a whole.

In the north basin of lake 302, it could be shown that the introduction of nutrients below the thermocline resulted in an ATP peak of biological activity at 9.0 m. Presumably this activity was mainly due to heterotrophic microorganisms as it was composed of material <10 μ and was not due to phytoplankton. However, the deep introduction of nutrients appears to have also affected shallower water layers. If the ATP data found in the enriched and unenriched basins are integrated from the surface to 8 m, it is found that the biomass of the enriched basin is approximately 2.5 times that of the unenriched basin. Much of this increase seems concentrated in water immediately above the enriched layer. A plausible explanation could be that a population of herbivores are feeding upon the microorganisms associated with the enrichment. Further work is now in progress to identify the species associated with the depths of high activity ATP concentration.

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Mr B. Townsend assisted us with the collection and extraction of ATP samples. Miss H. Kling provided phytoplankton counts and assisted in the interpretation of this data. Dr E. J. Fee kindly provided information regarding the storage of ATP samples. Miss M. Buckley identified zooplankton samples.

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Heterotrophic Utilization of Sucrose in an Artificially Enriched Lake

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Utilization of sucrose by heterotrophic microorganisms was measured in a small lake enriched with 5.54 g C/m² per year as sucrose. Heterotrophic activity exhibited fluctuations representing the damped oscillations of a disturbed steady state system, stabilizing 3 months after the commencement of enrichment. By late summer, reduction of the weekly addition of sucrose to a few micrograms per liter was accomplished within a day by the increased microbial activity resulting from rapid growth of heterotrophic microorganisms. Heterotrophic conversion (preenrichment) of sucrose to particulate carbon in the epilimnion between July and October 1971 was 2–17% of primary production. 25–35% of total sucrose utilized was converted to carbon dioxide, the remainder being incorporated into particulate material or released as nonvolatile products of metabolism.

THOMPSON, B. M., AND R. D. HAMILTON. 1973. Heterotrophic utilization of sucrose in an artificially enriched lake. J. Fish. Res. Board. Can. 30: 1547–1552.

Les auteurs ont mesuré l'utilisation du sucrose par des microorganismes hétérotrophes dans un petit lac enrichi de 5.54 g Cm²/année, sous forme de sucrose. L'hétérotrophie subit des variations, représentant les oscillations amorties d'un régime dont l'équilibre a été dérangé et qui se stabilise 3 mois après le début de l'enrichissement. A la fin de l'été, une réduction de l'addition hebdomadaire de sucrose à quelques microgrammes de sucrose par litre a été effectuée en une journée par une activité microbienne accure, résultat de la croissance rapide des microorganismes hétérotrophes. La conversion hétérotrophe (avant enrichissement) du sucrose en carbone particulaire dans l'épilimnion, de juillet à octobre 1971, a été de 2 à 17% de la production primaire. Du sucrose total utilisé, 25 à 35% a été converti en CO_2, la balance étant incorporée dans le matériel particulaire ou libérée sous forme de produits non volatiles du métabolisme.

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IN an effort to investigate the role of organic carbon in the eutrophication of Shield lakes, carbon, nitrogen, and phosphorus as sucrose, ammonium chloride, and ortho-phosphoric acid were added to lake 304 (Brunskill and Schindler 1971) located in the Experimental Lakes Area (Johnson and Vallentyne 1971). During 1971 enrichment proceeded at weekly intervals from May 26 to October 13 inclusive. Loading rates were 5.54 g carbon, 5.18 g nitrogen, and 0.4 g phosphorus/m² per year. While there are other extensive limnological investigations associated with this experiment (D. W. Schindler unpublished data), none is designed to evaluate the part played by heterotrophic microorganisms in the utilization of sucrose.

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Parsons and Strickland (1962) introduced a method for assessing the rate at which dissolved organic matter is transformed into particulate material by heterotrophic processes. Wright and Hobbie (1965) developed the technique further and, in response to suggestions in the literature (Hamilton and Austin 1966) Hobbie and Crawford (1969) expanded the method to include the measurement of dissolved organic carbon which is transformed into CO_2. The measurement of dissolved organic substances which are excreted as nonvolatile metabolites is still unsolved by this method. In spite of this deficiency the method appeared to offer a useful approach to the assay of heterotrophic utilization of sucrose added to lake 304.

In the investigation reported here we describe the kinetics of sucrose transformation in lake 304

during the period July 1971 to January 1972 and compare particulate carbon produced by heterotrophic processes with that produced by algal photosynthesis and furnished from other sources. A laboratory experiment designed to measure the disappearance of sucrose immediately after enrichment is described, and a comparison is made between the uptake kinetics of glucose and sucrose in lake 227 (enriched with nitrogen and phosphorus only) and lake 304.

Theory

The kinetics of utilization of low concentrations of substrate by heterotrophic microorganisms is frequently found to be analagous to those of simple enzyme–substrate reactions as described by the Michaelis–Menten equation:

$$v = \frac{V_m S}{K + S} \quad (1)$$

where v is the reaction velocity, V_m the maximum velocity attainable, and S the substrate concentration. K (a constant) is the substrate concentration at which $v = \frac{V_m}{2}$. One of the more rigorous linear transformations of the Michaelis–Menten equation is a modification of the Lineweaver–Burke equation (Dowd and Riggs 1965):

$$\frac{S}{v} = \frac{S}{V_m} + \frac{K}{V_m} \quad (2)$$

As:

$$v = \frac{dS}{D\mu t} \quad (3)$$

equation (2) can be transformed to read:

$$\frac{D\mu t}{d} = \frac{1.S}{V_m} + \frac{(K + S_n)}{V_m} \quad (4)$$

where D is the dpm from 1 μCi, μ the μCi added to that volume of the incubated sample which is filtered or used to estimate mineralization of substrate, t the incubation time in hours, d the dpm found in particulate material per volume filtered or as $^{14}CO_2$ per volume used to measure mineralization, S the substrate added, and S_n the naturally occurring substrate concentration, both in micrograms per liter. K is a transport constant and is considered to be a measure of the affinity of the population for substrate (Wright and Hobbie 1966). Data plotted in the form $\frac{D\mu t}{d}$ against S (equation 4) generate a linear plot with gradient $\frac{1}{V_m}$, x intercept $-(K + S_n)$, and y intercept T_t.

Kinetic parameters obtained from the utilization of organic nutrients can be interpreted in relation to a population of microorganisms. V_m gives information about the size of the microbial population (Wright and Hobbie 1966). A small V_m indicates the presence of few microorganisms capable of utilizing a particular substrate, while a high K indicates that relatively high substrate levels must be present before such populations could become effective competitors for the substrate. The value obtained for turnover time (T_t) of substrate already present in the water could be the result of several factors. A long T_t may result from a small adapted population of microorganisms in an environment containing high natural substrate levels, or simply an unadapted population. Conversely, a short T_t is observed when either a small adapted population and low natural substrate concentration or a large adapted population are present.

Materials and Methods

Uniformly labelled ^{14}C-sucrose (600 mCi/mM) and ^{14}C-D-glucose (288 mCi/mM) were obtained from Amersham/Searle (Arlington Heights, Ill.). Solutions of 1 μCi and 5 μCi/ml were made up in Super-Q (Millipore®) water, filter-sterilized, and aseptically dispensed into sterile acid-washed 10-ml glass ampules which were sealed and stored at –20 C. Unlabelled sucrose and D-glucose solutions were prepared in a similar manner such that 0.1 ml added to 50 ml of lake water resulted in final concentrations of 5 and 10 μg/liter. A sterile solution of 200 μg/liter sucrose was prepared for use in a laboratory enrichment experiment.

Samples were taken with either a 2-liter Van Dorn sampler or a battery-powered peristaltic pump. Prior to use the inside of the Van Dorn was scrubbed with 99% isopropanol and thoroughly rinsed with sterile lake water. The peristaltic pump was fitted with autoclaved silicone and surgical rubber tubing; integrated samples were taken above and below the thermocline, sampling from the surface down so as not to unduly disturb the water column. Samples were collected in autoclaved polypropylene bottles which were first rinsed out with a small amount of sample water. Large zooplankton were removed by filtering the water through an autoclaved 100μ stainless steel mesh which was, if reused, rinsed with a portion of the water sample prior to filtering. Samples were taken at the stations in lakes 227 and 304 representing maximum depth. All samples were transported to the laboratory in a backpack and processed within 1–1½ hr of sampling.

In the laboratory 50-ml subsamples were incubated in acid-washed, distilled water-rinsed, autoclaved glass

bottles containing 0.1 μCi of labelled substrate and increments of unlabelled substrate up to 40 or 50 μg substrate/liter. Incubations took place in the dark at $\pm$ 2 C of the in situ temperature, and lasted 1½–3 hr. Blanks at two or three substrate concentrations were fixed with 4 ml of neutral formalin and filtered at the end of the incubation time. This incubation procedure was repeated with a similar series of 100-ml serum bottles which were sealed with rubber serum stoppers immediately after addition of substrate.

Substrate incorporated into particulate material was estimated from 5 ml of incubated sample passed through a Millipore HA filter (0.45 μ pore-size) and rinsed with 2 × 5 ml of distilled water. The vacuum of less than ½ atmosphere was discontinued as soon as the water had passed through the filter, and the wet filter immediately dissolved in a dioxane-based fluor (Bray 1960 or Schindler 1966). $^{14}CO_2$ from samples incubated in serum bottles was collected in a scintillation fluor containing phenethylamine (Woeller 1961) by means of a closed-loop system incorporating a sealed push–pull diaphragm pump (Thompson and Hamilton 1973).

Laboratory enrichment with sucrose was performed upon a 6-liter integrated epilimnetic sample from lake 304. The sample was placed in a sterile 8-liter darkened glass aspirator bottle, stirred continually, and bubbled with sterile air for 5 min every half hour. At t = 0, 200 μg/liter of unlabelled sucrose was added to the vessel. This value was close to the concentration present just after enrichment assuming mixing throughout the epilimnion. At t = 0.25, 1.5, 3.0, 4.5, and 6.0 hr, series of 50-ml subsamples for measurement of uptake were taken (discarding the first 50 ml). One μCi of ^{14}C-sucrose was added to each subsample followed by increments of unlabelled sucrose up to 40 μg/liter. The vessel and incubated samples were kept within $\pm$ 0.5 C of the in situ temperature. All incubations were in the dark and lasted 1 hr. Further processing to determine uptake was performed as already described. The average velocity of sucrose uptake (SD = 3.2–12.8% of the mean) measured at each sampling time was used to calculate the sucrose removed from solution during that time interval.

Radioactive samples were counted to at least 1028 counts (SD = 3%) using a Picker Liquimat 220 liquid scintillation counter. Four vials containing 0.1 ml of the pertinent radioactive substrate were counted after each experiment to allow accurate calculation of specific activity. Conversion of cpm to dpm was accomplished using the external standard channels ratio technique (Bruno and Christian 1961).

An investigation into the replicability of the sampling and experimental techniques employed revealed that the standard deviation of dpm per 5 ml of incubated sample filtered expressed as a percentage of the mean was greater between replicate samples taken at the same sample station (13%) than between replicate samples taken from the same sample bottle (8.6%). Replicate 5-ml portions from the same incubated sample showed very little variation (SD = 1.4% of mean).

The following kinetic data relating to sucrose utilization in lake 304 between July 1971 and January 1972 are based on incorporation of sucrose–carbon into particulate material 1–21 hr prior to the weekly enrichment; the results were calculated on the basis of sucrose concentration. January data points were corrected for filtration error since uncorrected data were not statistically reliable. Any experimental data with a correlation coefficient of less than 0.9 when plotted according to equation (4) were rejected.

Results and Discussion

Kinetic Parameters

Values of V_m (Fig. 1A) were higher in the epilimnion than in the hypolimnion until fall overturn, indicating larger numbers of sucrose-utilizing microorganisms above the thermocline than below during summer. Fluctuations in epilimnetic V_m during July and early August levelled off from late August through September and October. Low values in November and January may have been due either to low water temperatures (1–2 C) hence reduced microbial activity, population decrease, or decreased adaptation of microorganisms following cessation of sucrose additions. Hypolimnetic V_m values increased during late September and October, probably due to mixing of the water column during fall overturn.

The turnover time (T_t) (Fig. 1B) of sucrose remaining in lake 304 prior to enrichment was most often shorter in the epilimnion than in the hypolimnion until mid-September. This may indicate greater microbial activity with respect to sucrose in the epilimnion although as already pointed out several elements contribute to the observed value of T_t. During this period epilimnetic T_t values averaged 7 hr, while hypolimnetic values were close to 14 hr. T_t throughout the water column decreased during late September and October, probably as a result of mixing of the water during overturn. During November and January the T_t of sucrose at 2 m increased by an order of magnitude over the October value. This might be expected owing to lowering of water temperature and corresponding reduction of microbial metabolic rates.

The ($K + S_n$) observed in the epilimnion was generally greater and more variable than in the hypolimnion (Fig. 1C). Since no measurement of S_n was made, the individual values of K and S_n are not known; measurement of S_n is expected in the near future. An increase in the January ($K + S_n$) over October and November values probably represents a population of microorganisms unadapted to sucrose since enrichment had ceased 3 months previously.

Filtration Error

On several occasions different volumes of incubated water sample were filtered and the filtra-

tion error calculated (Arthur and Rigler 1967). Neglecting filtration error could lead to underestimation of $(K + S_n)$ and V_m by up to a factor of 2 and overestimation of T_t by up to two thirds (Thompson 1973).

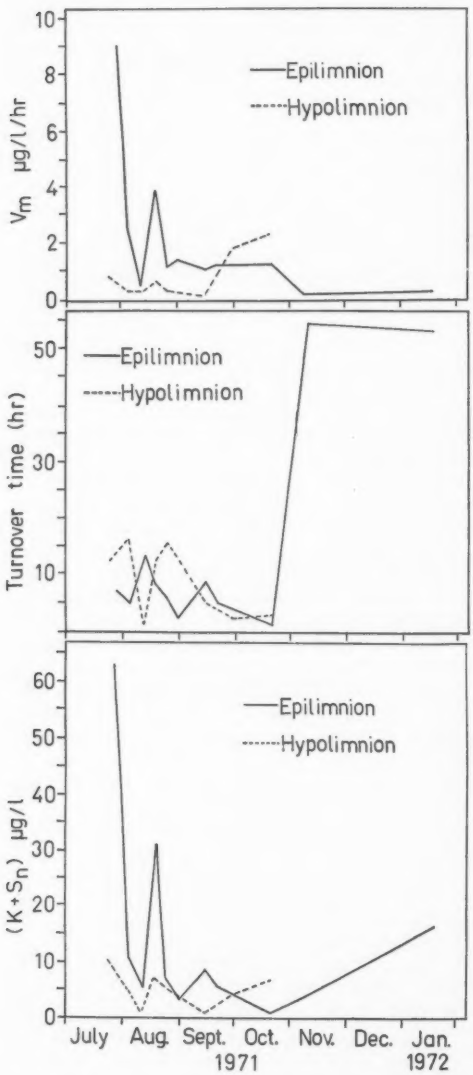

FIG. 1. Variation in A) V_m of heterotrophic microorganisms with respect to sucrose, B) turnover time (T_t) of sucrose remaining in the water column, C) $(K + Sn)$ with respect to sucrose. Lake 304, July 1971 to January 1972.

MINERALIZATION OF SUCROSE

Pertinent experiments showed that 25–35% of total sucrose utilized (i.e. mineralized and incorporated into particulate material) was converted to CO_2 by the heterotrophic microorganisms of lake 304. V_m is underestimated up to 1.8 times and T_t overestimated as much as 0.57 times if one neglects to measure mineralization of substrate. Values of $(K + S_n)$ corrected for mineralization vary between 0.67 and 1.26 times the uncorrected values (Thompson 1973).

THE FATE OF SUCROSE IN LAKE 304

On the basis of the distribution of Na^+ and NO_3^- added to lake 227 and Cl^- added to lake 304 (D. W. Schindler personal communication) it can be presumed that the added sucrose remained in the epilimnion of lake 304 and was utilized there. The presence of sucrose-utilizing micro-organisms in the hypolimnion may be due to sedimentation through the thermocline or transportation by migrating zooplankton.

The velocity of sucrose uptake after addition of 200 µg sucrose/liter to a sample of lake 304 epilimnetic water was found to increase exponentially with time up to 6 hr after enrichment (Fig. 2), indicating exponential growth of heterotrophic microorganisms. Assuming even mixing throughout the epilimnion, 266 µg sucrose/liter would have been present just after enrichment when the thermocline was at 3 m. Since 25–35% of total sucrose utilized was converted by the microorganisms to CO_2, the laboratory enrichment experiment of September 8 indicates that the weekly addition of sucrose to lake 304 would be reduced to a few micrograms per liter within a day. This data cannot be extrapolated to early summer since the microbial population may have been small or unadapted due to the schedule of sucrose additions.

The amount of sucrose–carbon converted to CO_2 by the microorganisms and thus added to the dissolved inorganic carbon (DIC) pool of the epilimnion of lake 304 was, on average, 30% of the total sucrose utilized. Therefore, 2.8 µM CO_2/liter/week was added to the epilimnetic DIC pool as a result of microbial utilization of 266 µg sucrose/liter per week. The DIC pool in the epilimnion of lake 304 during summer 1971 was generally equivalent to 10–20 µM CO_2/liter, dropping to 6 µM/liter at times (D. W. Schindler unpublished data). 2.8 µM CO_2/liter/week was therefore probably quite a

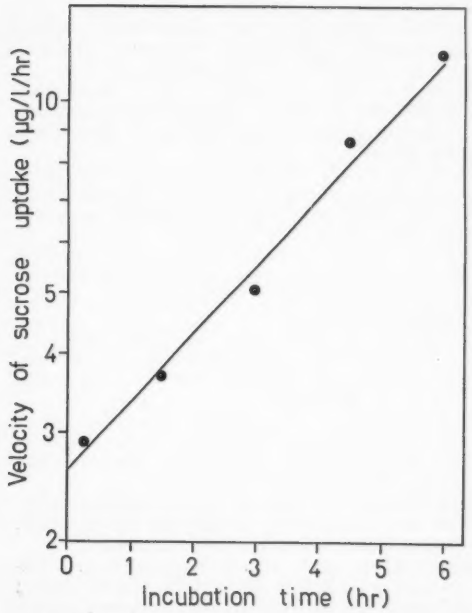

FIG. 2. Laboratory enrichment experiment, lake 304, September 8, 1971. Change in velocity of sucrose uptake with time after addition of 200 µg sucrose per liter.

small fraction of the total DIC available for primary productivity.

It is possible to compare preenrichment daily rates of primary production (calculated from the rate observed between 10 AM and 2 PM) with the rate at which sucrose is converted into particulate carbon by heterotrophic processes and the rate of total sucrose-carbon utilization. The kinetic parameters for sucrose uptake were corrected for filtration error and mineralization of sucrose. "S_n" was taken as $(K + S_n)$ which is an overestimation, especially at the beginning of the summer when K may have been large owing to poor adaptation of the heterotrophic microorganisms to sucrose. The velocity of utilization of sucrose at concentration $(K + S_n)$ was calculated and converted to mg C/m^2 per day down to 3 m and compared to primary production down to this depth (D. W. Schindler unpublished data).

Heterotrophic conversion of sucrose to particulate carbon from July to October 1971 was, on average, 8.2% of primary production. Conversion of sucrose-carbon to particulate material on seven out of nine occasions varied between 2 and 17% of carbon fixed by photosynthesis in the epilimnion.

Total sucrose-carbon utilization by heterotrophic microorganisms in the epilimnion of lake 304 represented a mean of 12% of carbon fixed by primary production, all but two of the values lying between 4 and 27%. However, since there is a burst of algal carbon fixation in lake 304 shortly after dawn similar to that observed in lake 227 (Schindler and Fee 1973; D. W. Schindler unpublished data), comparison of heterotrophic and photosynthetic carbon fixation during the middle of the day would lead to an overestimation of the relative role of heterotrophic microorganisms.

UTILIZATION OF D-GLUCOSE AND SUCROSE IN LAKE 227

Glucose utilization by heterotrophic microorganisms in lake 227 exhibits typical Michaelis–Menten uptake kinetics similar to those observed for glucose and sucrose utilization in lake 304 (Fig. 3). However, sucrose appeared to enter the microorganisms by a process more akin to diffusion. The observed $(K + S_n)$ values for sucrose utilization were 380 and 25 µg/liter at 0.5 and 2 m, respectively, while $(K + S_n)$ for glucose utilization at all depths measured was no greater than 5 µg/liter. These data indicate that the heterotrophic microorganisms in lake 227 are relatively unadapted to utilizing sucrose as a nutrient, and it can be inferred that sucrose is not a normal nutrient in this lake.

Changes in V_m (Fig. 1A) indicate that microbial activity fluctuated rather markedly during late July and most of August. These fluctuations appear to represent the damped oscillations of a perturbed steady state system. Probably, as in lake 227, sucrose was a relatively unimportant substrate for heterotrophic microorganisms in lake 304. The data

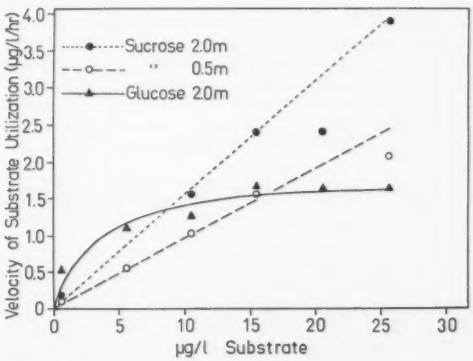

FIG. 3. Utilization of sucrose and D-glucose, lake 227, July 26, 1972.

indicate that the heterotrophic microorganisms in lake 304 reached a stable state with respect to the regime of sucrose additions about 3 months after the commencement of enrichment.

Wright and Hobbie (1966) suggested that V_m could be used as an index of eutrophication, a large V_m indicating a relatively greater level of eutrophication. Values of V_m representing sucrose utilization in lake 304 are of the same order of magnitude as V_m for glucose and acetate in two eutrophic Swedish lakes, lakes Norrviken and Lötsjön (Wright and Hobbie 1966).

After 1 year of enrichment with C, N, and P, lake 304 could be termed eutrophic (D. W. Schindler unpublished data). However, lake 227 reached a similar state after 1 year of enrichment with N and P only (D. W. Schindler et al. 1971). During the third year of enrichment it was found that lake 227 was obtaining almost 30% of its total carbon input from the invasion of atmospheric CO_2 (D. W. Schindler et al. 1972; Schindler and Fee 1973). The ratio of C to P added to lake 304 was greater than that normally found in sewage (Vollenweider 1968), yet total sucrose-carbon per m^2 added to lake 304 during 1971 was only one quarter of the amount calculated to enter lake 227 from the atmosphere. Although the sucrose added to lake 304 was effectively utilized by heterotrophic microorganisms and a portion of the carbon made available to the phytoplankton for primary production, this study indicates that an allochthonous source of organic carbon is not essential to the eutrophication of small Shield lakes.

Acknowledgments

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Effects of Cadmium and Copper on the Oxidation of Lactate by Rainbow Trout (*Salmo gairdneri*) Gills

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BILINSKI, E., AND R. E. E. JONAS. 1973. Effects of cadmium and copper on the oxidation of lactate by rainbow trout (*Salmo gairdneri*) gills. J. Fish. Res. Board Can. 30: 1553–1558.

The oxidative activity of gill filaments was determined by measuring the liberation of $^{14}CO_2$ from Na-lactate-3-^{14}C. In fish surviving the exposure to 10^{-5} M $CdCl_2$ (1.12 mg Cd/liter) for 24 hr or to 10^{-6} M $CuCl_2$ (0.064 mg Cu/liter) for 48 hr, the oxidation of lactate by gills was inhibited by over 50%. The exposure of trout to lower metal ion concentrations resulted in mortalities, but there was no detectable effect on the oxidative activity in gills.

BILINSKI, E., AND R. E. E. JONAS. 1973. Effects of cadmium and copper on the oxidation of lactate by ranbow trout (*Salmo gairdneri*) gills. J. Fish. Res. Board Can. 30: 1553–1558.

Nous avons déterminé l'activité oxydante des filaments branchiaux en mesurant le $^{14}CO_2$ libéré du Na-lactate-3-^{14}C. Chez les poissons survivant à l'exposition à 10^{-5}M $CdCl_2$ (1.12 mg Cd/litre) pendant 24 h ou à 10^{-6} $CuCl_2$ (0.064 mg Cu/litre) pendant 48 h, l'oxydation du lactate par les branchies diminue de plus de 50%. L'exposition de truites arc-en-ciel à de plus faibles concentrations d'ions métalliques cause des mortalités mais n'a pas d'effet notable sur l'activité oxydante des branchies.

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THE purpose of the present study was to determine whether or not the exposure of rainbow trout, *Salmo gairdneri*, to copper and cadmium salt solutions results in a significant inhibition of lactate oxidation in gills. It was recently reported that trout gills show a comparatively high ability to oxidize lactate to CO_2 (Bilinski and Jonas 1972) and histopathological evidence for the damage of fish gills by heavy metals was presented in several studies. Baker (1969) observed changes in gill architecture after copper poisoning of winter flounder, *Pseudopleuronectes americanus*, and Gardner and Yevich (1970) detected histopathological changes in gills of mummichog, *Fundulus heteroclitus*, exposed to cadmium. Although the toxicity of cadmium and copper to fish has received considerable attention in recent years, the effects of these metals on the oxidative metabolism in gills have not been reported.

Materials and Methods

EXPOSURE OF FISH TO HEAVY METAL SOLUTIONS

Rainbow trout (*Salmo gairdneri*) were obtained from Sun Valley Trout Farm, Mission, B.C. and maintained under conditions described earlier (Bilinski et al. 1971). The fish used in the toxicity tests had a mean weight of 129.0 g (SD ±24.1, 230 fish). The trout were exposed to solutions of heavy metal salts, as described in the standard static bioassay procedure of the American Public Health Association (1965). Fiberglass tanks (180 liters) without metal parts were used to expose four fish. They were supplied with city water (Vancouver–Capilano River system) which has been dechlorinated with activated carbon. The total hardness, measured by E.D.T.A. method (BDH kit), was 4 mg $CaCO_3$/liter. Water temperature was maintained at 10–11 C by circulating well water through a tygon tube inserted at the bottom of the tanks. Every 24 hr the exposed fish were introduced into fresh solutions of toxicants and the control fish were transferred to tanks containing fresh water. Salts used to prepare the test solutions were $CuCl_2 \cdot 2H_2O$ and $CdCl_2 \cdot 2\frac{1}{2}H_2O$ (Fisher certified reagents).

DETERMINATION OF THE OXIDATIVE ACTIVITY IN GILL FILAMENTS

Gill arches were cut individually from fish, killed by decapitation, and rinsed under a gentle stream of aquarium water to eliminate blood deposits. Excess water was removed by blotting with filter paper. Gill filaments were cut with a scalpel and weighed directly in Warburg flasks, using a precision balance. All operations were carried out at 3–5 C. The oxidative activity in gill filaments was determined by measuring the liberation of

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$^{14}CO_2$ from Na-lactate-3-^{14}C according to the procedure described by Bilinski and Jonas (1972). The techniques employed for diffusion and recovery of $^{14}CO_2$ and determination of radioactivity by scintillation counting were the same as described previously (Bilinski and Jonas 1970). Unless otherwise stated, all assays on gill tissue were conducted under the following standard incubation conditions: 50 mg of the terminal portion of gill filaments (about one-third of the total length of filaments); substrate, Na-lactate-3-^{14}C, 5 mM; incubation time, 2 hr at 15 C.

Determination of Cd and Cu Content of Gills

The determinations were made using 300-mg samples (wet tissue) as starting material. Cadmium was measured by the dithizone procedure of Smith et al. 1955. Copper was determined by the diethyl-dithiocarbamate method as described by Strickland and Parsons (1968).

Histological Techniques

Immediately after killing the fish, specimens of gill tissue were excised, fixed in Bouin-Holland-Sublimate, and embedded in Paraplast. Sections of the gill were cut at a thickness of 5 μ and stained with Mayer's hematoxylin and eosin (Culling 1957).

Results

The experiments reported in Table 1 and Fig. 1 and 2 were conducted to develop a standard procedure for the determination of lactate oxidation by gill tissue in vitro. Initial trials showed that the terminal part of gills consisting of well-separated filaments had greater oxidative activity than the gill tissue adjacent to the cartilaginous arches but there was no significant difference in the activity of filaments originating from the different arches (Table 1). The oxidative activity in gill filaments was readily depressed by homogenization or by cutting them into small pieces (Table 1). When 50 or 100 mg of filaments were used, a constant relationship existed between the incubation time and the oxidative activity during a 3-hr incubation period at 15 C (Fig. 1). In the presence of 50-mg tissue the oxidative activity reached a maximum rate at 5 mM concentration of lactate (Fig. 2).

In experiments on the toxicity of cadmium to trout, live fish were exposed for 4–96 hr to $CdCl_2$

Table 1. Oxidative activity in various preparations of gill filaments.

Tissue preparation	Oxidative activity[a]
Terminal portion of filaments	1.297±0.083(6)[b]
Gill tissue adjacent to arches	0.327±0.042(6)
Terminal portion of filaments from 1st arch	1.223±0.149(3)[c]
Terminal portion of filaments from 2nd arch	1.470±0.172(3)
Terminal portion of filaments from 3rd arch	1.398±0.055(3)
Terminal portion of filaments — homogenate	0.278±0.025(3)
Terminal portion of filaments — finely cut tissue	0.494±0.045(3)

[a] mμmoles Na-lactate-3-^{14}C converted to $^{14}CO_2$/g wet tissue/hr; standard incubation conditions.
[b] Mean value, standard error, and number of tests.
[c] The levels of activity in arch 1, 2, and 3 are not significantly different (Student's *t*-test).

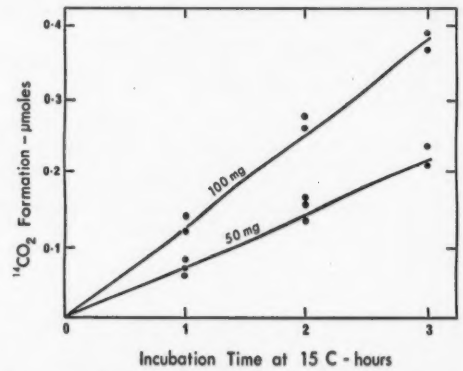

Fig. 1. Effect of incubation time and amount of tissue on the oxidation of Na-lactate-3-^{14}C by gill filaments. Other conditions are the same as in the standard incubation procedure.

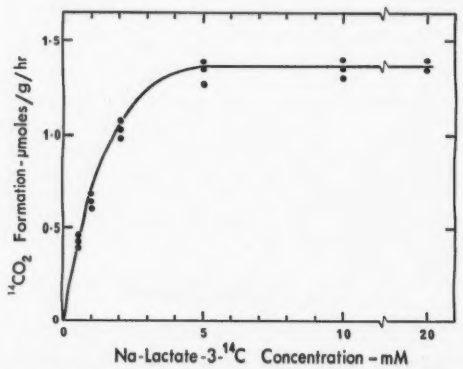

Fig. 2. Effect of substrate concentration on the oxidation of Na-lactate-3-^{14}C by gill filaments; standard incubation procedure.

solutions at concentrations ranging from 10^{-4} M (11.2 mg Cd/liter) to 10^{-7} M (0.0112 mg Cd/liter) (Table 2). At 10^{-4} M concentration, $CdCl_2$ was extremely toxic to trout and all fish died within 7 hr of exposure. In gill filaments originating from fish surviving a 4-hr exposure to 10^{-4} M $CdCl_2$, the oxidative activity was inhibited by 57%. Trout exposed to 10^{-5} M $CdCl_2$ for 24 hr had a mortality rate ranging from 33 to 50% and the oxidation of lactate was significantly depressed (50–63% inhibition) in gills from surviving fish. When trout were exposed to 10^{-6} M $CdCl_2$ there were no mortalities after 24 hr but 17% of fish died after 48 hr and 66% after 72 hr. In fish surviving a 48–72 hr exposure there was no significant change in the ability of gill tissue to oxidize lactate. A 96-hr exposure of trout to 10^{-7} M $CdCl_2$ also had no detectable effect on the oxidative activity in gill filaments though the mortality rate was high.

A similar series of experiments was conducted on the toxicity of copper to trout using $CuCl_2$ solutions at concentrations ranging from 10^{-5} M (0.636 mg Cu/liter) to 10^{-7} M (0.0064 mg Cu/liter) (Table 3). At 10^{-5} M concentration, $CuCl_2$ was more toxic to trout than $CdCl_2$ since all fish died within 24 hr. There were no mortalities in trout exposed to 10^{-6} M $CuCl_2$ for 24 hr. After 48 hr the mortalities amounted to 25–50% and in the gills of surviving fish the oxidative activity was significantly depressed (53–61% inhibition). A 96-hr exposure of trout to 10^{-7} M $CuCl_2$ caused mortalities (33%) but there was no significant change in the oxidative activity in gills from surviving fish.

The accumulation of Cd and Cu in gills of fish exposed to these metals was measured under conditions that showed inhibition in vivo (Table 4). The cadmium introduced into the 2-ml incubation medium with a 50-mg gill sample from fish exposed for 24 hr to 10^{-5} M $CdCl_2$ amounted to 0.6 μg. The addition of up to 2.00 μg Cd to the incubation medium had no effect on in vitro oxidative activity in control fish. The copper present in a 50-mg sample from fish exposed for 48 hr to 10^{-6} M $CuCl_2$ amounted to only 0.005 μg and the addition of up to 5.0 μg Cu to the incubation medium did not affect the measurement of oxidative activity in control fish. It was found that comparatively high levels of cadmium (20 μg) or copper (50 μg) were needed to produce in vitro inhibition of lactate oxidation by gill filament (Table 4).

Several samples of gills in which the oxidation of lactate was partially inhibited in vivo were

TABLE 2. Effects of the exposure of trout to Cd^{++} on the oxidative activity in gill tissue.

Treatment of fish[a]				Oxidation of lactate by gill filaments			
Expt. no.	$CdCl_2$ concn. (M)	Duration of exposure (*Hr*)	Mortalities (%)	Control fish (*Oxidative activity*)[b]	Treated fish	Significance level (*P*)[c]	Inhibition (%)
1	10^{-4}	7	100	–	–	–	–
2	10^{-4}	4	0	1.256[d] ±0.088(4)	0.537 ±0.079(4)	<0.01	57
3	10^{-5}	24	33	1.287 ±0.129(3)	0.470 ±0.023(3)	0.01	63
4	10^{-5}	24	50	1.277 ±0.109(6)	0.637 ±0.115(6)	<0.01	50
5	10^{-6}	24	0	–	–	–	–
6	10^{-6}	48	17	1.162 ±0.074(3)	0.978 ±0.141(3)	NS[e]	–
7	10^{-6}	72	66	1.255 ±0.174(4)	1.305 ±0.107(4)	NS	–
8	10^{-7}	96	75	1.221 ±0.040(3)	1.290 ±0.260(3)	NS	–

[a] In each experiment 12 fish were exposed to the solutions of metal chlorides under conditions described in Material and Methods. Four fish, used as controls, were maintained for the same duration in toxicant-free water. There were no mortalities in the control group.

[b] mμmoles Na-lactate-3-^{14}C converted to $^{14}CO_2$/g wet tissue/hr; standard incubation conditions.

[c] Student's *t*-test.

[d] Mean value, standard error, and number of fish tested.

[e] No significant difference.

examined histologically (Fig. 3). Only one out of five fish exposed to 10^{-6} M $CuCl_2$ for 48 hr showed changes in gill structure (Fig. 3(2)). This alteration involved a detachment of the epithelial layer in a number of lamellae. In fish exposed to 10^{-5} M $CdCl_2$ for 24 hr extensive degeneration of gill structure was noted (Fig. 3(3)). The most conspicuous changes were detachment of epithelial layer from the remaining parts of the lamellae and the hypertrophy and hyperplasia of the interlamellar epithelium. Lesions, while focal in nature, often made structural analysis difficult due to accumulation of cell debris.

Discussion

The oxidative activity of gill tissue was determined in the present study by measuring the formation of $^{14}CO_2$ from Na-lactate-3-^{14}C. Such a transformation reflects a complete oxidation of the

TABLE 3. Effects of the exposure of trout to Cu^{++} on the oxidative activity in gill tissue.

Treatment of fish[a]				Oxidation of lactate by gill filaments			
Expt. No.	$CuCl_2$ conc. (M)	Duration of exposure (*Hr*)	Mortalities (%)	Control Fish (*Oxidative activity*)[b]	Treated Fish	Significance level (*P*)[c]	Inhibition (%)
1	10^{-5}	24	100	–	–	–	–
2	10^{-6}	24	0	1.251[d] ±0.127(5)	1.299 ±0.207(5)	NS[e]	–
3	10^{-6}	48	25	1.199 ±0.129(3)	0.471 ±0.105(3)	0.05	61
4	10^{-6}	48	50	1.105 ±0.196(3)	0.517 ±0.106	0.05	53
5	10^{-6}	48	33	1.217 ±0.065(4)	0.475 ±0.045(4)	<0.01	61
6	10^{-7}	96	33	1.125 ±0.102(5)	1.120 ±0.061(5)	NS	–

[a,b,c,d,e]For explanation, see footnotes Table 2.

TABLE 4. Cd and Cu content of gills and its effects on oxidative activity in vitro.

Preparation	In gill filaments Cd (*μg/g wet tissue*)	In gill filaments Cu (*μg/g wet tissue*)	In incubation mixture (2 ml) Cd (*μg*)	In incubation mixture (2 ml) Cu (*μg*)	Oxidative activity % of control
A — Filaments from fish exposed for 24 hr to $CdCl_2$ 10^{-5} M[a]	12.8	–	0.64		50[b]
B — Filaments from fish exposed for 48 hr to $CuCl_2$ 10^{-6} M[a]	–	0.100	–	0.005	39[c]
C — Filaments from control fish[a]	<1.0	<0.01	–		100
— Filaments from control fish[a] with addition of $CdCl_2$[d]	–	–	0.20–2.00	–	96–105(9)[e]
— " " " " "	–	–	20.00	–	71–78(3)
— Filaments from control fish[a] with addition of $CuCl_2$[d]	–	–	–	0.05–0.50	95–103(9)
— " " " "	–	–	–	5.00	93–99(3)
— " " " "	–	–	–	50.00	68–80(3)

[a]Standard incubation conditions.
[b]See Experiment No. 4, Table 2.
[c]See Experiment No. 5, Table 3.
[d]Filaments were preincubated for 20 min at 2–5 C in presence of the metal salts.
[e]Range and number of tests.

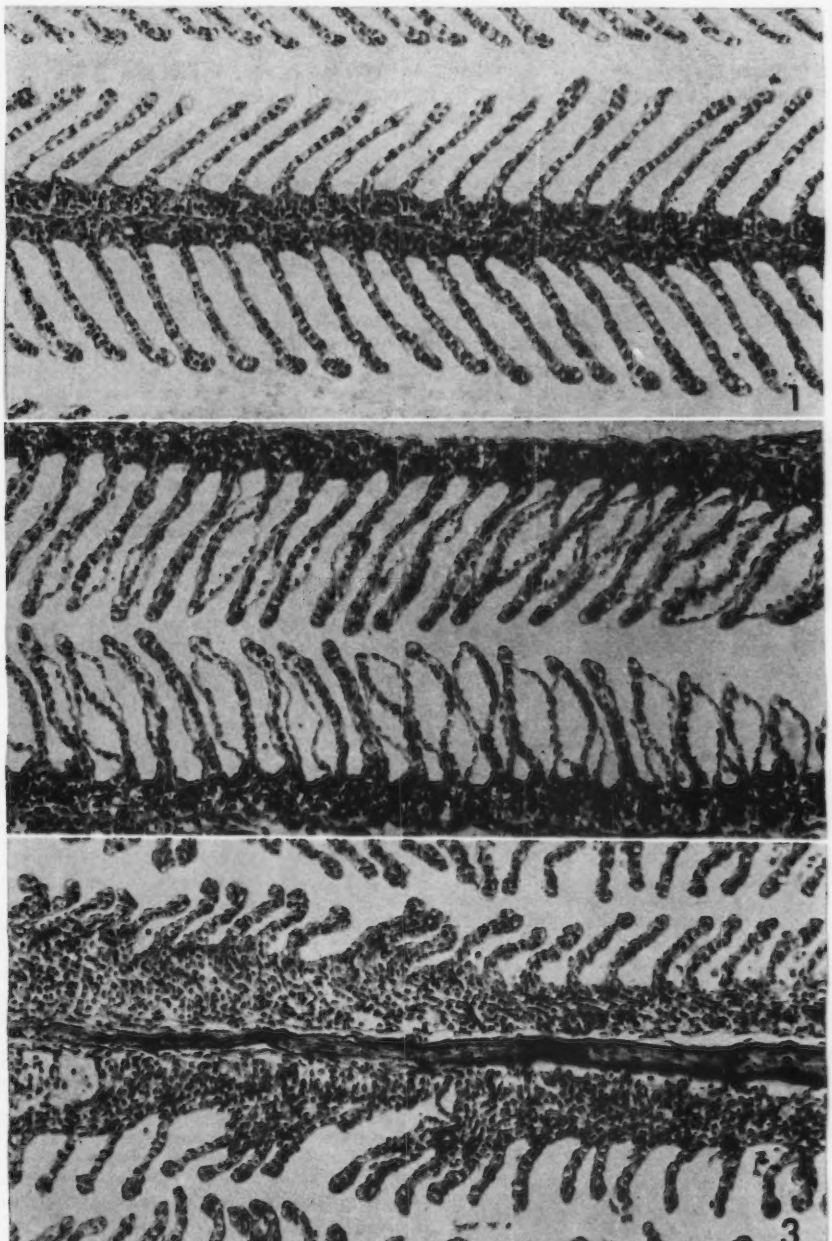

FIG. 3. Gill filaments. (1) depicts the normal structure; (2) shows detachment of the lamella epithelium of fish exposed to 10^{-6} M $CuCl_2$ for 48 hr; (3) exhibits the fusion of the lamellae and the accumulation of cell debris in the basal lamellar region of fish exposed to 10^{-5} M $CdCl_2$ for 24 hr (×100).

carbon skeleton of lactate and it involves the conversion of lactate to pyruvate and acetyl coenzyme A followed by the oxidation of the latter in mitochondria under the action of enzymes of the tricarboxylic acid cycle (Bilinski and Jonas 1972). The impairment of this process in gills of fish exposed to $CdCl_2$ of $CuCl_2$ might be due to disruption of the cellular organization or to inhibition of enzymic activity. Since gills from trout exposed to 10^{-5} M $CdCl_2$ for 24 hr showed pronounced histopathological changes, it is reasonable to assume that the disruption of cellular structure was the primary cause of the inhibitory effect. The exposure of trout to 10^{-6} M $CuCl_2$ for 48 hr was generally not associated with histopathological changes and it appears likely that under these conditions the inhibition of lactate oxidation was due to the interference of metal ions with the enzymic activity.

Heavy metals are known to inhibit enzymes requiring sulfhydryl groups in the expression of activity (Hewitt and Nicholas 1963). Jackim (1973) found that the exposure of mummichog, *Fundulus heteroclitus* to copper (10 mg/liter) resulted in reduction of liver δ-aminolevulinate dehydrase activity. Hubschman (1967) showed that in vitro copper (10^{-5} M) depresses the oxidation of succinate by crayfish, *Orconectes rusticus*, hepatopancreas. The exposure of crayfish to 5 mg Cu/liter for 48–72 hr resulted in inhibition of succinate oxidation in hepatopancreas but no reduction in oxidative activity was detected in animals exposed to lower copper concentrations. Cross et al. (1970) found that, in vitro, cadmium (5×10^{-3}M–5×10^{-4}M) inhibits the respiration of pulmonary alvealor macrophage cells and mitochondria from sheep. Jackim et al. (1970) studied the effects of heavy metals on five liver enzymes (alkaline and acid phosphatase, catalase, xanthine oxidase, and ribonuclease) from *Fundulus heteroclitus*. They found that the changes in enzyme activity produced by the exposure of live fish were not necessarily of the same magnitude or direction as those observed when the metals were introduced into the enzyme preparations. In the present study the accumulation of copper and cadmium in gills from the exposed fish was determined and it was found that the addition of comparable amounts of these metals to gill preparations from control fish had no effect on the oxidative activity. Higher concentrations were needed to obtain the inhibition in vitro. These differences could be attributed to the slow rate of penetration of copper and cadmium into the cells of intact gill filaments.

The cause of death of fish exposed to copper or cadmium does not appear to be well established. It has been suggested that the death of fish in acute poisoning by heavy metals is due to the disruption of the respiratory processes caused by the damage to gill epithelium (Skidmore 1970; Eisler 1971; Burton et al. 1972). The deleterious effects of copper and cadmium to fish are manifested by changes in blood composition (McKim et al. 1970; Gardner and Yevich 1970), damage to kidney, liver, intestines, cephalic lateral canals, and olfactory systems (Baker 1969; Gardner and Yevich 1970; Gardner and Laroche 1973), alterations in oxygen consumption (O'Hara 1971), reduction in growth and reproduction (Pickering and Gast 1972), and abnormal behavior performance (Mount and Stephan 1969).

It appears from the present study that the test for lactate oxidation had a value only at high exposure levels to copper and cadmium. Since this technique could serve for detecting damage to fish gills in general, it might find an application with other toxic agents.

Acknowledgments

The authors wish to thank Mr J. R. McBride from this laboratory for making the histological examinations of gills. The valuable assistance by Mr Y. C. Lau in conducting the bioassays is gratefully acknowledged.

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Description and Distribution of New Specimens of the Fish *Lipogenys gilli* from the Western North Atlantic

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TEMPLEMAN, W. 1973. Description and distribution of new specimens of the fish *Lipogenys gilli* from the western North Atlantic. J. Fish. Res. Board Can. 30: 1559–1564.

Nine specimens of the rare fish *Lipogenys gilli* Goode and Bean, 1895, previously known from a single specimen taken off Maryland in 1887, were taken between 1957 and 1970 along the continental slope of the Nova Scotian Shelf to southwestern Grand Bank, at depths of about 400–800 m. These specimens, 238–375 mm in standard length, have a remarkably low variation in vertebral number (228–234). From the additional specimens the description of the species is considerably extended.

TEMPLEMAN, W. 1973. Description and distribution of new specimens of the fish *Lipogenys gilli* from the western North Atlantic. J. Fish. Res. Board Can. 30: 1559–1564.

Neuf spécimens du rare poisson *Lipogenys gilli* Goode et Bean, 1895, connu jusqu'à maintenant par un seul individu capturé au large du Maryland en 1887, ont été capturés entre 1957 et 1970 le long du talus continental, du plateau néo-écossais à la partie sud-ouest du Grand banc, à des profondeurs d'environ 400–800 m. Ces spécimens, mesurant 238–375 mm de longueur standard, ont un compte vertébral accusant remarquablement peu de variation (228–234). Ces exemplaires additionnels ont permis d'ajouter considérablement à la description.

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Lipogenys gilli, the only species of the family Lipogenyidae, order Notacanthiformes, was described (Goode and Bean 1895, 1896) from a single specimen taken off Maryland, USA. The description of the holotype is incomplete. The authors provided approximate relative body proportions rather than exact measurements, a good general description, excellent figures, and only a few of the possible meristic counts.

Nine additional specimens, six males and three females, were taken along the continental slope off Nova Scotia to southwestern Grand Bank and used as the basis for a more detailed description of the species. The specimens, 238–375 mm in standard length, are all smaller than the holotype, 43 cm in total length. At least the three females were very immature.

The new specimens are in the collection of the St. John's Station.

[1]Present address.

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Materials and Methods

The nine specimens of *L. gilli* described here were obtained by research vessels of the St. John's Biological Station of the Fisheries Board of Canada in bottom otter-trawling for redfish between 1957 and 1970. They were originally preserved in 10% formalin and later at some time before measuring transferred to 50% isopropyl alcohol.

Unless stated otherwise, the methods of measurement and of meristic counting were those of Hubbs and Lagler (1958). Apart from total lengths and standard lengths, which were the shortest distances between perpendicular parallel planes, measurements were direct distances between points.

When vertebrae were fused, each component of a fused group was counted as one (Templeman 1970).

In the anal fin, anteriorly there are definite spines without annulations, followed by spines with traces of annulations, then by spine-rays with increasing numbers of annulations until they become flexible and ray-like, but still possessing sharp spine-like tips (Fig. 3). Abruptly these change into definite rays with brush-like tips of fine setae. The change from spine-like tips to brush-like tips forms a natural and useful division in counting anal fin elements.

In the dorsal fin (Fig. 4) a similar transition from true spines to true rays occurs within a smaller number of elements. The dorsal begins anteriorly with a few simple spines without annulations. The first spine can usually be detected externally but occasionally is below the skin. Radiographs were used to detect whether or not a hidden first spine was present. Passing posteriorly, the spines become more complex; traces of annulations begin and

TABLE 1. Records of *Lipogenys gilli*.

Spec. no.	Date	Locality	Lat. N Long. W	Depth (*m*)	Standard length (*mm*)	Sex	Bottom temp. (*C*)	Bottom temp. Depth (*m*)
1.	May 23, 1970	SW slope Grand Bank	43°29′30″ 52°01′30″	350–505	330	M	4.84	395
2.	Oct. 21, 1962	SW slope Grand Bank	43°59′00″ 52°42′30″	395–565	311	M	3.78	457
3, 4.	Oct. 10, 1957[a]	S slope Green Bank	44°56′ 54°50′	440–585	297;367	F,M	4.10	547
5, 6.	Nov. 22, 1962	E slope Sable I. Bank	43°29′40″ 59°48′00″	595–840	238;375	F,F	4.16	732
7.	Nov. 14, 1959	S slope Emerald Bank	42°51′30″ 62°31′00″	400	297	M	4.51	549
8.	Nov. 12, 1959	S slope Emerald Bank	42°49′15″ 62°39′00″	640–705	298	M	4.39	750
9.	Nov. 12, 1962	Slope off LaHave Bank	42°39′30″ 64°14′30″	450–610	350	M	4.43	732
10.	Sept. 17[c] 1887[b]	Off Maryland	37°46′30″ 73°56′30″	1582	Ca 430[d]	–	3.28[c]	–

[a]These specimens were taken by the research vessel *Investigator II*. All specimens 1959–70 were taken by the *A. T. Cameron*.
[b]No. 39212 U.S.N.M. (Goode and Bean 1895). Taken by *Albatross* large beam-trawl.[c]
[c]From Smith (1889).
[d]17 inches, presumably total length.

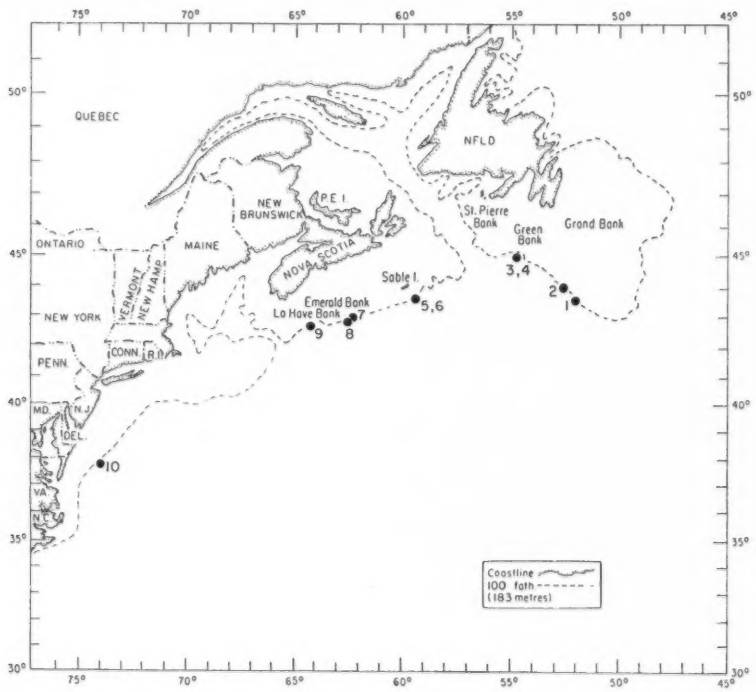

FIG. 1. Locations of capture of *Lipogenys gilli* (numerals are specimen numbers from Table 1).

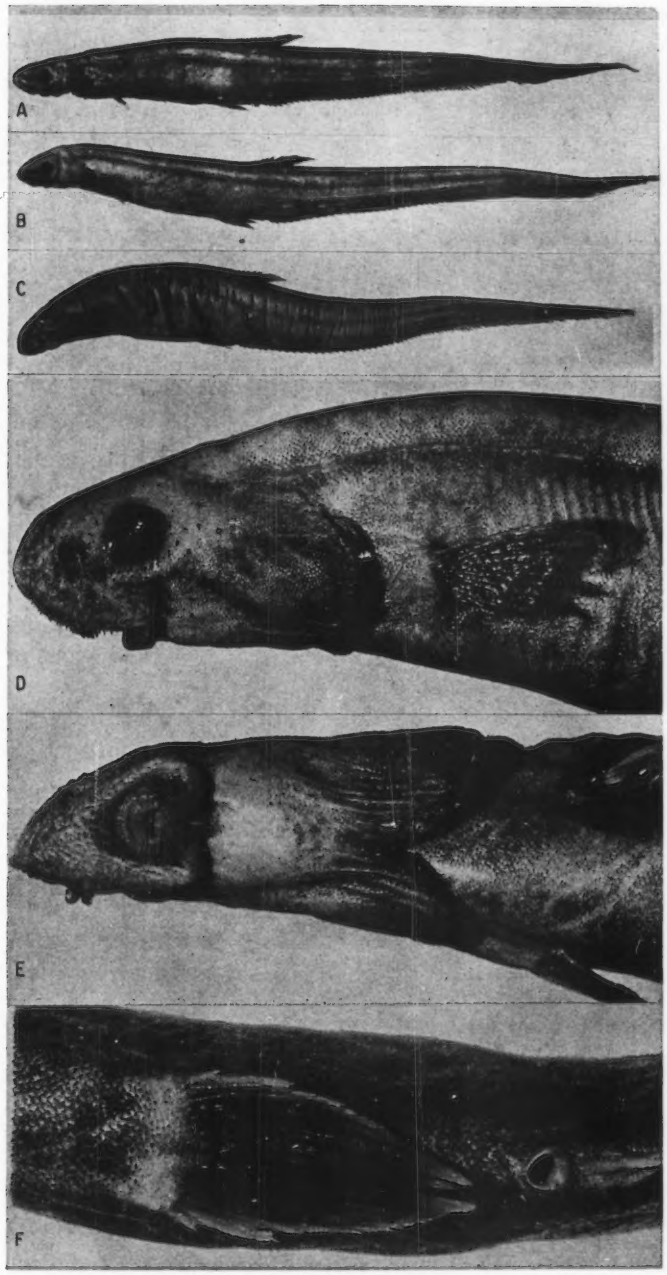

FIG. 2. *Lipogenys gilli*. A–C: lateral views of specimens 8, 1, and 4 (Tables 1, 2); A, 298 mm SL; B, 330; C, 367. The fish are typically straight, the curves being due to preservation in small containers. D (specimen 4): lateral view of head and anterior part of body showing velum and lateral line; E (specimen 4): ventral view of head showing mouth and medially divided lower jaws; F (specimen 1): ventral view of pelvic fin in relation to cloaca.

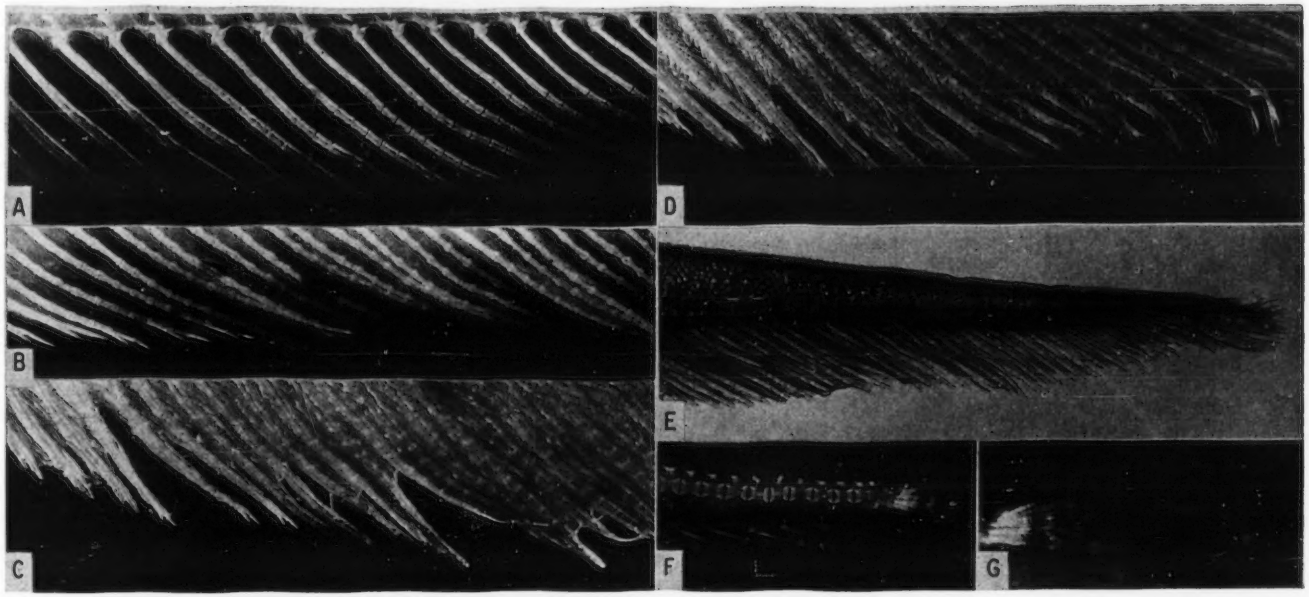

FIG. 3. *Lipogenys gilli*. Spines and rays of anal fin, and caudal fin. A (specimen 1)–D (specimen 7): change from true spines without annulations to true rays with brush-like tips of setae. A: gradually increasing number of annulations and articulations and increasing flexibility in spine–rays with pointed tips. B and C (specimen 1), D: the change, slightly posterior to position of A, from spine-rays with sharply pointed tips to true rays. E (specimen 4): posterior part of anal fin, and caudal fin. F (specimen 1): 9 posterior vertebrae, urostylar half vertebra, and posterior rays of anal fin. G (specimen 1): base of caudal fin with five rays. A, B, F, and G are from radiographs. The most posterior spine–ray tip included in A is the first to be included anteriorly in B.)

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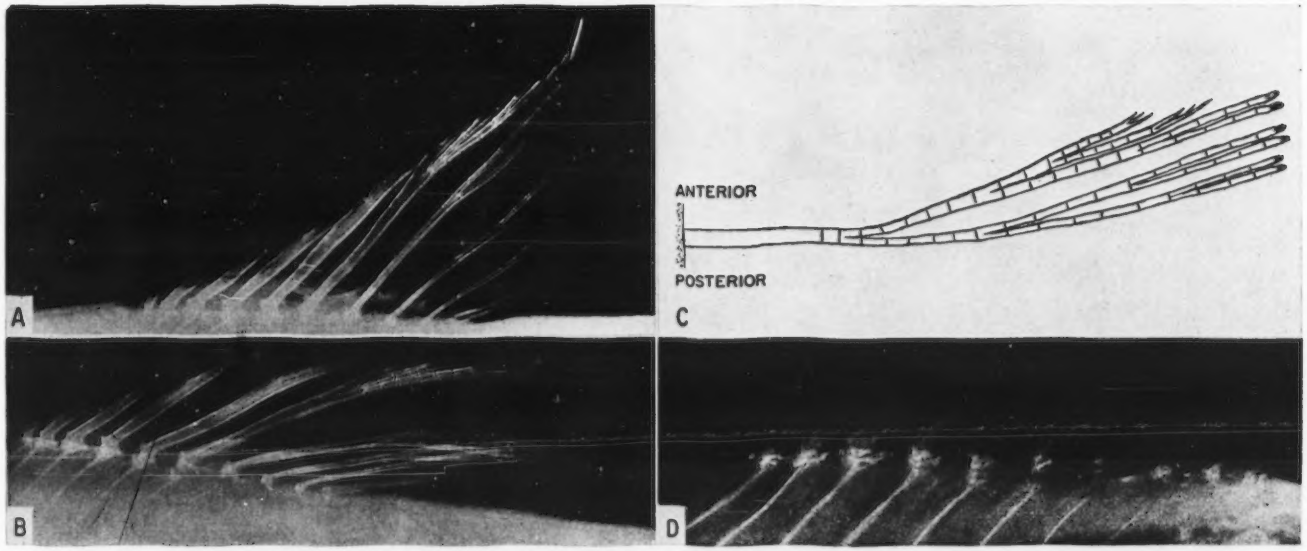

FIG. 4. *Lipogenys gilli*. Radiographs and drawing of elements of dorsal fin. A and B: elements 1–5, spines without distinct annulations (but note smaller spines on distal anterior face of fifth); 6, a spine-ray with well-articulated posterior branches but all with sharp spine-like tips; 7, a composite spine-ray; 8–11, true rays. Because of lack of calcium, partly due to original preservation in formalin, the finer spine and ray tips often cannot be seen. C: lateral view of posteriormost spine-ray (element 7 of A and B) to show anterior sharp spine-like tips and posterior typical ray-like brush tips of fine setae. D: radiograph of base of dorsal fin showing closeness but distinctness of bases of posterior two rays. (A–C, specimen 1; D, specimen 5).

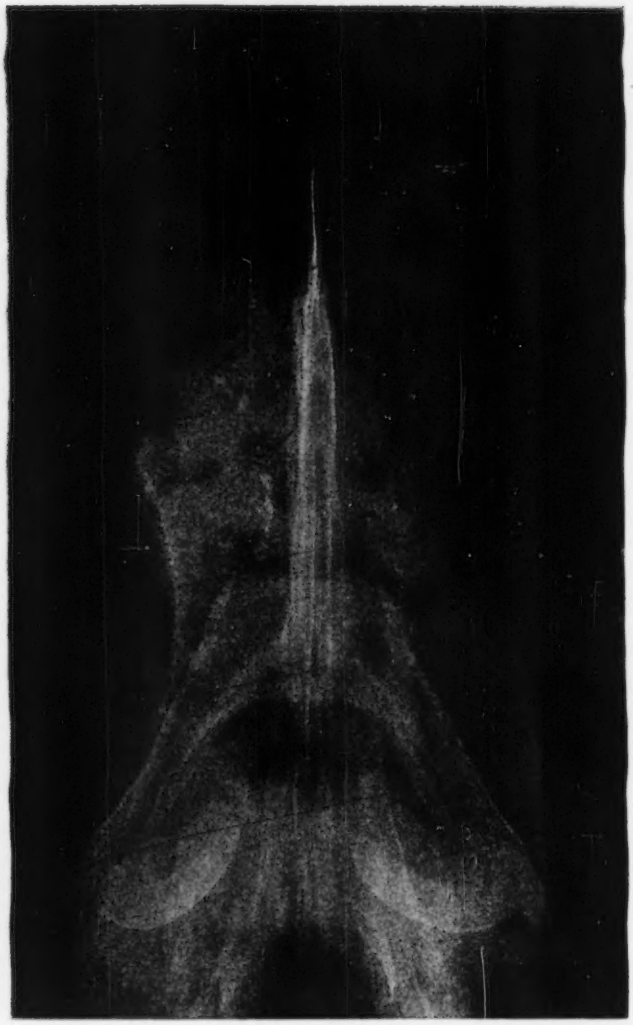

FIG. 5. *Lipogenys gilli*. Radiograph (ventral view) of anterior part of head (A, specimen 8) showing jaws separated posteriorly and concealed spine at angle of jaw.

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short sharp spine-like branches appear along the anterior distal edge followed by a branched spine–ray with faint or no annulations anteriorly and with well-segmented ray-like branches posteriorly (element 6 of Fig. 4A and B). Apart from the exceptions noted below, the branches of this element all have sharp spine-like tips. The next element posteriorly is branched and well-segmented throughout. Usually this element but sometimes the previous one (but only one of them in a dorsal fin) has anterior branches with sharp spine-like tips and posterior branches with brush-like tips of fine setae. I have called this element the composite spine–ray (Fig. 4C). The branches of all the more posterior elements have brush-like tips of fine setae and are true rays. The composite spine–ray forms a useful division point for meristic counting, providing, as in the somewhat similar division of the anal, additional characters for study of variation between specimens from different areas. It is evident from their figures and numbers of rays that Goode and Bean counted the posterior two rays as one; because they appear to come from separate bases (Fig. 4D), I have counted them as two.

Distribution

The new specimens (Table 1, Fig. 1–2) were taken from shallower water than the holotype (about 400–800 m versus 1582 m). They extend the range northward and eastward along the edge of the Nova Scotian Shelf to southwestern Grand Bank. On two occasions, two specimens were taken in one set. No additional specimens were obtained in many research otter-trawling sets from 1958 to 1972 at depths of 730 m and shallower, at about 90-m intervals, along the continental slope from Baffin Island to Georges Bank.

Meristic Characters

The known meristic characters of the holotype are within the ranges of these characters in the new specimens (Table 2).

TABLE 2. Meristic characters of *L. gilli*.

Spec. no. (Table 1)	Pectoral fin rays[a]	Pelvic fin spines and rays[b]	Anal fin spines and rays			Dorsal fin spines and rays			Vertebrae[e]	Lateral-line pores[f]	Pyloric caeca	Dorsal spine under which anal begins	Vertebra from post. end under which anal ends[g]	Number of vertebrae fused	Sex
			Spine tipped	Ray tipped[c]	Total	Spine tipped[d]	Ray tipped[c]	Total							
1	12	III,7	52	76	128	7	4	11	232	135	6	5	3	0	M
2	12	III,7	52	–[h]	–[h]	7	4	11	234	125	–[h]	5	–[h]	3	M
3	13	III,7	46	71	117	8	4	12	233	128	5	3	5	2	F
4	12	III,7	55	80	135	7	4	11	234	129	6	4	2	4	M
5	14	III,8	48	88	136	6	5	11	228	–[h]	6	1	2	0	F
6	12	III,6	44	78	122	7	5	12	233	129	6	4	3	4	F
7	12	III,7	49	67	116	8	4	12	233	122	5	4–5	5	0	M
8	13	III,8	50	80	130	7	5	12	230	122	7	4	4	0	M
9	12	III,7	58	–[h]	–[h]	8	4	12	–[h]	121	7	1	–[h]	–[h]	M
10[i]	–	III,7	51	78	129	–	–	11[j]	–	–	–	4	–	–	–

[a]Usually of left fin, unless unsuitable.

[b]The first spine is not always visible without dissection. Counts are of left pelvic; right pelvic has same number of spines and rays as left except that no. 6 and 8, have seven rays.

[c]With brush-like tips of fine setae. Last two rays counted separately.

[d]Up to and including the composite spine–ray with anterior spine-like tips and posterior ray-like (brush-like) tips.

[e]From radiographs. Urostylar half-vertebra included as one vertebra.

[f]Includes count of pore systems from in front of gill cleft to the point where the lateral line disappears posteriorly, over the region where the outline of the lateral-line canal is apparent externally.

[g]The vertebral number at which the anal fin ends does not include the urostylar half-vertebra.

[h]Unsuitable for this count.

[i]From Goode and Bean (1895, 1896).

[j]Increased by one to allow for counting last two rays as two. (Caudal rays were not always distinct, but appeared to be five in number.)

The meristic characters show only narrow ranges for pectoral rays (12–14), pelvic spines (3), and rays (6–8), dorsal spines + rays (11–12), vertebrae (228–234), and pyloric caeca (5–7). The numbers of anal fin spines (44–58), rays (67–88), and totals of these (116–136), and of lateral-line pores or pore systems (121–135), are considerably more variable.

The anal fin in each case is complete anteriorly and apparently complete posteriorly. Posteriorly each anal ray is approximately opposite a vertebra (Fig. 3F) and the range in the vertebral number from the posteriormost vertebra at which the anal fin ends (only 2–5) is not great enough to account for differences as great as 21 in anal-spine + fin-ray number.

In the composite spine–ray of the dorsal fin (Fig. 4C), the numbers of anterior spine-like and ray-like tips of the branches (but especially the first of these) are highly variable between individuals (4–27 spine-like and 2–8 ray-like) and within the same individual (from 27 spine-like and two ray-like through 7 and 7 to 4 and 8). Usually the separation of sharp tips and brush-like tips within the composite spine–ray is distinct. In the composite spine–ray of only one specimen was a spine-like tip noted in the dorsal after the brush-like tips began, and in this case only one sharp spine tip immediately posterior to the first brush-like tip.

No gill rakers were noted in the one specimen dissected for this purpose (No. 6 of Tables 1 and 2). No latitudinal, longitudinal, or sex differences in meristic characters are apparent. The lowest number of lateral-line pores and the highest number of pyloric caeca are in the most southern and western localities, but these may be chance occurrences.

A small range in vertebral number and lack of significant differences in vertebral averages over wide areas were noted by Templeman (1970) for the Greenland halibut, and this may be typical of many deepwater fishes living in water of approximately the same temperature over wide ranges. Even when, as in the Greenland halibut, the eggs are pelagic, they may not float to the surface but be in an intermediate layer. As fin-ray numbers are determined later in development than vertebral numbers, they may be more subject to the more variable upper layer temperatures, if the larvae in later development are higher in the water column.

The problem of the transition and nomenclature of rigid and annulated or articulated spines and

TABLE 3. Morphometric ratios in the holotype and new specimens of *L. gilli*.

Body part ratio	Holotype (from Goode and Bean 1895, 1896)	New specimens Range (avg)
Height/width	ca. 2	2.0–2.5(2.3[a])
Length/height	ca. 10(9.4[b])	8.2–12.5(10.3)[c]
Length/head length	8 2/3(8.5)	7.7–8.8(8.2)[d]
Origin pectoral–cloaca/head length	ca. 2	1.8–2.5(2.0)
Head length/eye diameter	ca. 5(5.0)	4.8–5.5(5.1)
Head length/snout length	ca. 4(3.5)	3.0–3.4(3.2)
Postorbital length/snout length	ca. 2(1.9)	1.6–1.9(1.8)
Eye diameter/mouth diameter	ca. 2(3.7)[e]	3.3–5.0(4.0)
Interorbital width/eye diameter	ca. 1	0.8–1.2(1.0)
Snout–dorsal/head length	ca. 3(3.2)	3.0–3.6(3.2)
Head length/length dorsal base	ca. 2(2.0)	2.3–2.5(2.4)
Snout–pelvic/head length	ca. 2.5(2.7)	2.5–2.9(2.6)
Posterior end dorsal — post. end lateral line/head length	ca. 2.5(2.5)	2.2–2.6(2.4)
Pectoral length/postorbital length	little > 1	1.0–1.2(1.1)

[a,c]For all specimens, though one is excluded for these measurements in Table 4 (footnote b).

[b]Values in parentheses calculated from figure of *L. gilli* in Goode and Bean (1895) except as noted. Length = total length; head length and postorbital length measured to the end of bony operculum; eye = cornea; snout measured to beginning of cornea and postorbital from end of cornea. New specimens measured similarly except length is standard length.

[c,d]On basis of total length, ratios (averages) for c and d are 8.4+ to 12.9+ (10.6+) and 8.0+ to 9.0+ (8.4+).

[e]Mouth diameter from figure in Goode and Bean (1896).

rays and the occasional formation of spines from segmented rays was discussed, especially for the related notacanths, with particular reference to *Notacanthus bonapartei*, by Marshall (1962).

Morphometric Characteristics

All the new specimens show the general characteristics of the genus and species: the roundish, inferior, suctorial mouth with the imperfect lower jaw separated posteriorly and medially (Fig. 2E, 5), the concealed spine at the angle of each jaw (Fig. 5), and the other characteristics described by Goode and Bean. The one specimen dissected in detail No. 6 of Tables 1 and 2 has no teeth.

The ranges of ratios of body proportions of the new specimens are in general close to or include the values attributed to the holotype (Table 3).

The figures of *L. gilli* in Goode and Bean (1895, 1896) appear to be in correct proportion. Because only total length was mentioned by these authors and not standard length, the former was probably used and from the ratios calculated from their figures, their other methods of measurement were probably similar to mine. Calculations from these figures often bring the approximate ratios attributed

TABLE 4. Morphometric characteristics of *L. gilli*. (Except where indicated otherwise, all body parts are expressed in percentages of standard length. Specimen numbers examined are no. 1–9 of Table 1, 6 male and 3 female.)

Item no.	Body part	Range of body proportions (average)
1	Total length (greatest) (*mm*)	246+–385+ (326+)
2	Standard length: snout to base of caudal (*mm*)	238–375(318)
3	Head: length, snout to end bony operculum	11.4–13.0(12.2)
4	Head: length, snout to end velum	12.2–13.8(13.0)
5	Cornea: horizontal diameter	2.3– 2.6(2.4)
6	Interorbital width: least fleshy[a]	2.1– 3.0(2.5)
7	Snout: length to anterior border cornea	3.6– 4.2(3.8)
8	Postorbital: length cornea to end bony operculum	6.5– 7.1(6.7)
9	Postorbital: length cornea to end velum	7.1– 7.7(7.4)
10	Mouth: transverse diameter circular opening	0.5– 0.8(0.6)
11	Fold around mouth: transverse diameter to outside of fold	1.5– 1.9(1.7)
12	Snout to mouth	3.7– 4.7(4.3)
13	Body: greatest height	8.0–10.0(9.4)[b]
14	Body: greatest width	3.9– 4.6(4.2)[b]
15	Dorsal fin: length base	4.7– 5.5(5.1)
16	Dorsal fin: length longest element	5.6– 8.2(6.6)
17	Anal fin: length longest ray	2.5– 4.2(3.4)
18	Pectoral: length	6.5– 7.5(6.9)M 7.8– 8.3(8.0) F
19	Pelvic: length	6.1– 6.7(6.4) M 6.9– 7.9(7.3) F
20	Snout to anterior base 1st spine dorsal fin	36.9–40.8(38.7)
21	Snout to dorsal base pectoral fin	14.2–15.2(14.7)
22	Snout to anterior base anal fin	37.8–41.9(40.0)
23	Snout to beginning of pelvic fin	30.5–33.7(32.2)
24	Anterior dorsal origin pectoral to mid-cloaca	22.8–28.0(24.8)
25	Posterior dorsal base to posterior end lateral line	27.5–31.2(28.9)
26	Tip pelvic fin to anterior border cloaca[c]	–0.2 to 2.2 (1.0)M –1.3 to 0.0(–0.7) F
27	Tip pelvic fin to base 1st anal spine	0.8– 3.1(1.9) M 0.4– 1.4(0.7) F

[a]From above mid cornea.

[b]Unusual specimen no. 4 (Table 1) — height 12.2%, and width 4.9% — not included in range or averages. Including this specimen the averages are: greatest height 9.7, greatest width 4.3.

[c]Minus values represent the distance (%) that the pelvic fin extends posterior to the anterior border of the cloaca.

to the holotype even closer to the averages of the ratios for the new specimens. The only serious difference is in the eye diameter: mouth diameter ratio, which for the holotype is only half as large as the average for the new specimens. When this ratio is calculated from the figures of Goode and Bean, however, it is well within the range of the new specimens (Table 3).

The pelvic fin of the holotype reaches the cloaca. In three of my nine specimens (Table 4), the pelvic fin extends beyond the anterior border of the cloaca, in another it reaches the anterior border and in the remaining five it does not reach the cloaca (Fig. 2F). Three of the four in which the pelvic fin reaches as far as the cloaca are the three females.

Within the nine specimens, ranging only from 238 to 375 mm in standard length, the body proportions (Table 4) are not highly variable and no very definite trends with fish length are apparent. It is possible that pectoral and pelvic fin lengths are relatively greater in females but no definite statements can be made on the basis of the small numbers and the small range of lengths examined. One fish, No. 4 (Fig. 2C; Table 4, footnote b), is much stouter — and especially has a much greater body height — than the others, but its testis is small and its other measurements are well within the range of the remaining specimens.

All the new specimens have a definite caudal fin (Fig. 2, 3) and a complete vertebral column. There is no evidence of loss of any part of the caudal or truncation of the caudal with formation of a new caudal fin as is so common in the grenadiers and notacanths.

Acknowledgments

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Bioassay Procedures to Evaluate Acute Toxicity of Neutralized Bleached Kraft Pulp Mill Effluent to Pacific Salmon

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DAVIS, J. C., AND B. J. MASON. 1973. Bioassay procedures to evaluate acute toxicity of neutralized bleached kraft pulp mill effluent to Pacific salmon. J. Fish. Res. Board Can. 30: 1565–1573.

A series of bioassays was carried out to assess the acute toxicity of neutralized, filtered, bleached kraft pulp mill effluent (BKME) from a single mill to underyearling Pacific salmon in fresh soft water, at 10–13 C. Toxicity expressed in terms of the 96-hr LC50 varied from 22% of full strength BKME to nontoxic in different collections. A procedure is described for estimating the 4-day LC50 from geometric mean survival time data. Toxicity of effluents changed unpredictably with storage (even at 2 C), and declined with air stripping. Comparison of continuous flow and static test procedures indicated that continuous flow procedures yield somewhat higher toxicity results than static tests. Experiments with varying fish densities indicate that measurable toxicity is less in static tests with heavy fish loading. Use of loading densities of 2.5 liters/g fish or better is recommended. Young sockeye salmon (*Oncorhynchus nerka*) appeared most sensitive, and pink (*O. gorbuscha*) and coho (*O. kisutch*) salmon somewhat more resistant to toxic BKME solutions. No correlation was found between time to death and condition factor in the size range of underyearling coho tested (3.0–7.3 cm). Recommendations are made for routine and regulatory bioassay procedures.

DAVIS, J. C., AND B. J. MASON. 1973. Bioassay procedures to evaluate acute toxicity of neutralized bleached kraft pulp mill effluent to Pacific salmon. J. Fish. Res. Board Can. 30: 1565–1573.

Nous avons effectué une série d'analyses biologiques en vue de déterminer la toxicité aiguë de l'effluent neutralisé, filtré et blanchi d'une seule usine de pâte à papier kraft (BKME) à l'endroit de saumons du Pacifique de moins d'un an dans de l'eau douce fraîche à 10–13 C. La toxicité, exprimée en termes de LC50 après 96 h, varie de 22% de concentration maximale de BKME à nulle pour différentes collections. Nous décrivons une méthode pour estimer rapidement la LC50 après 4 jours, à partir des moyennes géométriques du temps de survie. La toxicité des effluents change d'une façon qui ne peut être prédite au cours de l'entreposage (même à 2 C) et diminue avec le stripage à l'air. Une comparaison d'essais à flot continu et d'essais statiques démontre que le procédé à flot continu résulte en une toxicité quelque peu plus élevée que les essais statiques. Des essais avec diverses densités de poissons indiquent que la toxicité mesurable est moindre dans les essais statiques avec fortes densités de poissons. Des densités maximales de 2.5 litres/g de poissons ou plus sont recommandées. Les jeunes saumons nerka (*Oncorhynchus nerka*) semblent les plus sensibles, et les saumons roses (*O. gorbuscha*) et coho (*O. kisutch*) quelque peu plus résistants aux solutions toxiques de BKME. Nous n'avons pu établir de corrélation entre le temps à mourir et l'état d'embonpoint dans la gamme de longueur des saumonneaux coho soumis aux essais (3.0–3.7 cm). Nous recommandons certaines procédures d'analyses biologiques routinières et orientées vers l'établissement de normes.

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WASTE from bleached kraft pulp mills is a common pollutant across Canada. Kraft mill wastes are mixtures of chemicals, containing known and unknown toxicants and are highly variable in effect on fish. The need for standardized bioassay procedures for regulatory and experimental purposes is great. Recently, federal regulations for pulp mill emissions and guidelines for toxicity testing were drawn up and are currently undergoing revision. Hopefully these findings will be helpful in formulation of regulations and procedures and can be generally applied to studies of other toxicants.

Although extensive bioassays have been carried out with kraft pulp mill effluents (Alderdice and Brett 1957; Betts and Wilson 1967; Hicks and DeWitt 1971; Howard and Walden 1965, 1971,

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1972; Servizi et al. 1966; Servizi and Gordon 1973; Sprague and McLeese 1968a, b; Werner 1963) there is poor documentation of the interaction of toxicity of kraft wastes with factors such as fish size and age; sample test volumes, effluent treatment and storage, temperature and aeration procedures. In the course of using bleached kraft mill effluent (BKME) for sublethal physiological studies (Davis 1973) the experiments reported here were conducted to study the relation of acute toxicity with some of these bioassay factors.

Methods

Underyearling coho salmon, *Oncorhynchus kisutch* (40–110 mm, 0.5–15 g), sockeye salmon, *O. nerka* (40–90 mm, 1–10 g), and pink salmon, *O. gorbuscha* (35 mm, 0.2–0.3 g) were used. All fish were hatched from eggs in gravel-filled hatchery troughs and reared in 2000-liter fiberglass tanks on natural photoperiod with oxygen levels at air saturation. Temperature in the holding tanks was 9.5–11 C year round and between 10–13 C in all bioassays. Temperature during any one bioassay did not vary more than 0.5 C. Well water of 15–20 ppm hardness was used for rearing and all bioassays. Some small sockeye whose size was reduced by restricted ration and holding at 6.5 C were also used, but were acclimated to test conditions and fed normally for several weeks prior to use. None of the fish were fed for the duration of the tests.

Bioassay vessels were all-glass aquaria of varying capacity with smoked plexiglass lids. Convenient tank liners were constructed of nylon netting stretched over stainless steel wire frames. These liners could be lifted up to visually inspect the fish in each tank and ensured that all fish received identical treatment during inspection. Aquaria were immersed in a temperature-controlled bath and lightly aerated with a small airstone.

Effluent was obtained from a full bleach kraft mill with a production capacity of 540 ADT (Air dry tons — US) per day of full bleach pulp. Water discharge from the process is 50,000 US gal/ton pulp produced, soda loss 100 lb/ton, fiber loss 15 lb/ton. The bleaching sequence is CEDED (chlorination, caustic extraction, chlorine dioxide, caustic extraction, chlorine dioxide) and 130 lb/ton chlorine is used in full bleach production. No treatment is practised and alkaline (including caustic extraction effluent) and bleach plant acidic wastes are sewered separately. Wood pulp is usually a chip mixture of 25% western red cedar, 30% Douglas fir, and 45% hemlock. Samples were collected only when mill personnel felt the process was operating in a normal, trouble-free fashion. Collection took approximately ½ hr on a given day and consisted of pumping grab samples from the mill sewers.

Effluent samples of 200–400 gal were transported immediately to the laboratory where the alkaline and combined bleach wastes were stored separately at 2 C in closed containers. Sixteen hours prior to testing the solutions were combined in the proportion released from the mill, thus approximating a composite effluent emission. This effluent consisted of 70% alkaline sewer waste (including caustic extraction effluent), 18% chlorination effluent, 7.5% 1st chlorine dioxide effluent, and 4.5% 2nd chlorine dioxide effluent. Usually the composite effluent was aerated for 16 hr, neutralized with 0.5 NaOH or H_2SO_4, then filtered through pyrex glass wool. The rationale behind neutralization and air stripping was that this permitted study of the residual toxicity of the effluent, free from the effect of *p*H or large amounts of volatile toxicants. No precipation of material was evident upon neutralization.

Recording and Treatment of Data

Mortality was recorded by regularly lifting the net basket in each tank and observing the fish through several inches of test solution. The criterion of death was cessation of respiratory movements. Frequency of observations varied although in most instances bihourly checks of each tank were conducted on a 24-hr basis.

Two data treatment procedures were employed. The first, recommended by Sprague (1969) was determination of the 4-day LC50, the concentration lethal to 50% of the fish in 96 hr. First individual times to death of fish were plotted on log-probability paper, for several test concentrations and a line estimate for each concentration was eye-fitted (Fig. 1A). It has been shown (Litchfield and Wilcoxon 1953) that log-probit lines fitted by eye are usually highly accurate. The median lethal time was read directly from the line for each concentration and plotted on log–log paper, relating it to BKME concentration (Fig. 1B). The value of the asymptote to the time axis is the approximate value of the incipient LC50. A more rapid technique of estimating median response time useful for strong BKME solutions is the calculation of geometric mean survival time (GMST), an accurate estimate of the median (by definition) of regular log-normal data (Brown et al. 1967; Sprague 1969; Shepard 1955). This alternate estimate may be used in place of the graphically estimated values (Fig. 1B), and has also been used for comparative purposes throughout this paper. It is calculated (Steel and Torrie 1960) by:

$$\text{GMST} = (T_1 \times T_2 \text{ --- } \times T_n)^{\frac{1}{n}}$$

where T_1 = time (min) to first death and T_n = time (min) to second to last death. As the time to zero mortality does not enter into the calculation, time to 100% mortality is not used either to keep the distribution uniform (Shepard 1955).

Special variations of these general methods will be described where relevant in the Results section.

Results

Preaeration of Effluent

Aeration generally reduces kraft mill effluent toxicity and is a recognized method of effluent

treatment. Sulphur compounds are oxidized and lower toxicity may result (Werner 1963). Volatiles such as methyl mercaptan can be air-stripped from the effluent (Hynninen 1971). Oxidation or air-stripping of a highly toxic component could greatly reduce toxicity of an effluent or, conversely, if small quantities of volatile toxicant(s) were present in the first place, little change might result from aeration. This factor may account in part for the observed variability in BKME toxicity, and its sensitivity to aeration and storage.

To test the influence of aeration on toxicity a composite sample of BKME was divided in half and one portion was aerated for 16 hr while the other was tightly stoppered. Both samples were tested identically and aerated during the bioassay. Results (Table 1) show that toxicity was generally reduced by aeration, but in varying degrees. In four cases toxicity was markedly reduced, but in four other cases there was little or no noticeable effect. In no cases, however, was there increased toxicity after aeration. In 96-hr static bioassays using preaerated solutions of BKME, most mortality occurred in the first 48 hr. These results suggest declining effluent toxicity with time which may be related to oxidative phenomena or other factors described herein.

Flow-Through Procedures

Static and continuous flow procedures were compared using the same effluent and equal fish densities. Flow-through systems consisted of glass tanks with side arm overflows and 13-gal Mariotte bottles (Engstrom-Heg 1971) providing a solution replacement rate of 90% in 13 hr (Sprague 1969). This flow rate was 5.52 ± 0.2 liters/hr into 30-liter tanks. Effluent was neutralized, filtered, and diluted to the test concentration with well water prior to filling the Mariotte bottles.

Toxicity in static situations appeared somewhat less than in flow-through situations even if the solution in the flow-through system had been air-stripped (Table 2). Variability between test results is likely partly due to the presence of volatile toxicants and their rate of elimination from solution. The Mariotte bottle may also be a poor choice of dosage apparatus for BKME work with non-airstripped samples as, by nature of the operation of the air intake tube, some air bubbles through

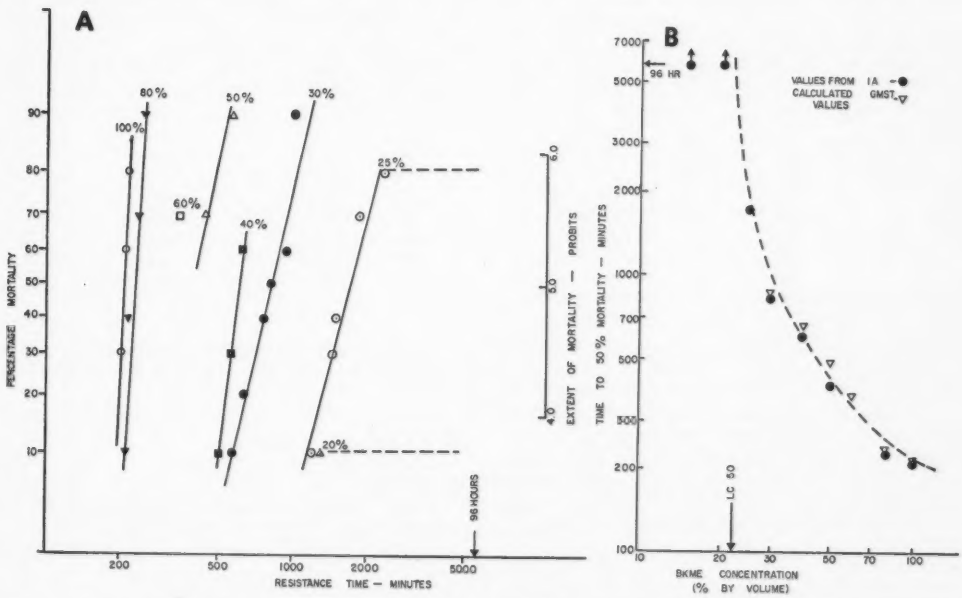

FIG. 1. A, Cumulative mortality data for underyearling coho in static bioassays at various BKME concentrations. BKME concentrations in % full strength appear with each line. Data are for a single batch of effluent; B, A comparison of time to 50% mortality estimated from the probit plot in Fig. 1A (circles) and that calculated as the geometric mean survival time (triangles) for each concentration.

the effluent in the bottle. Similarly, care must be taken to insure that fresh effluent flowing from the Mariotte bottle into the test tank enters below the surface of the solution in the tank to minimize air-stripping of the introduced sample.

Fish Density in Test Tanks

Groups of underyearling sockeye salmon (*Oncorhynchus nerka*) were used in differing densities in solutions of equal toxicity to illustrate the relation between GMST and density. The results (Fig. 2) clearly show that there is a direct relation between density and geometric mean survival time. That is, the higher the weight to volume ratio, the higher the value of GMST. Only at solution volumes 2.5 liters/g or above was no difference in GMST detected. Clearly, relatively large solution volumes (>2.5 liters/g) are required to eliminate effects of crowding on survival time. Howard and Walden (1972) also stressed the importance of loading density on BKME bioassay results.

The relation is not constant over a range of toxicities. For each effluent of different toxicity, GMST's were determined over a range of fish to volume ratios, and the results were plotted (Fig. 3). The slope of each line was calculated assuming the GMST to be the dependent variable. The slope of the weight to volume relation provides an index of the degree of toxicity. A steep slope suggests a solution of low toxicity, while a shallow slope accompanies a highly toxic solution. Obviously a small change in the weight to volume ratio will have little effect on toxicity test results with highly toxic solutions, but could have a large effect on solutions of low toxicity. Conversely slight variations in fish weight would drastically affect the results of tests with low toxicity BKME samples.

Species Comparisons

Examples of bioassays designed to test the comparative sensitivities of pink, sockeye, and coho underyearlings to BKME are given in Table 3.

Table 1. Some examples of toxicity of preaerated and nonpreaerated BKME solutions to underyearling coho salmon. Oxygen levels were maintained at 80–100% saturation and temperatures were 10±.5 C. Dilution water was well water containing 20 ppm total hardness as $CaCO_3$.

LC50 of preaerated BKME (%v/v)	Concn BKME %v/v	Preaerated			Nonpreaerated			Procedure used	Wt range of fih s(g)
		liters soln./ g fish	no. killed	GMST (*min*)	liters soln./ g fish	no. killed	GMST (*min*)		
100	100	1.43	1/10	–	1.35	10/10	896	static	.5–1.4
	75	1.43	0/10	–	1.96	6/10	–	static	.4–.7
75	100	1.00	10/10	1364	.89	10/10	1470	static	.6–1.0
	75	1.00	2/10	–	.97	3/10	–	static	.6–.8
60	100	.495	10/10	825	.46	10/10	815	static	4–9
38	75	.66	6/6	626	.63	6/6	536	static	7–10
	40	–	0/6	–	.57	6/6	1319	static	8–11
	40	.53	6/6	1245	–	6/6	1008	cont. flow	8–11

Table 2. Some examples of comparative toxicity of BKME samples tested with static and continuous flow procedures using underyearling coho salmon. Oxygen levels were maintained at 80–100% saturation and temperatures were 10±.5 C. Dilution water was well water with 20 ppm total hardness as $CaCO_3$.

LC50 of preaerated static BKME	Concn BKME %v/v	Static			Continuous flow			Sample preaerated	Wt range pf fish (g)
		liters soln./ g fish	no. killed	GMST (*min*)	liters soln./ g fish	no. killed	GMST (*min*)		
60	60	.5	2/5	–	.5	5/5	1829	yes	5–10
	40	–	0/5	–	.5	2/5	–	no	5–10
38	33	.43	5/5	1275	.44	5/5	1075	no	6–8
	40	.57	6/6	1175	.5	6/6	1500	no	6–10
	40	–	0/6	–	.53	6/6	1245	yes	6–10
	40	.57	6/6	1319	.5	6/6	1008	no	6–8

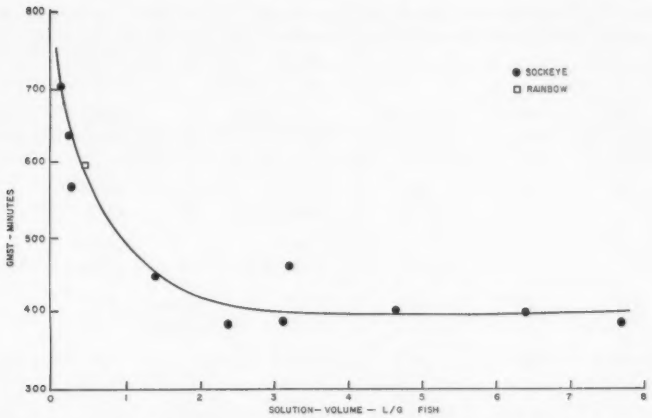

FIG. 2. GMST's for groups of sockeye underyearlings (0.7–1.3 g, 5–8 fish/tank) and a group of rainbow trout (2.3–4.2 g, eight fish) tested at different solution loading densities. All fish were in 75% full strength BKME (2.5 × the LC50) at 10 C in soft fresh water. Effluent was previously air-stripped for 20 hr and neutralized. Tests were done in static tanks with gentle aeration and no solution replacement.

Comparisons indicated sockeye were generally more sensitive to BKME than coho, when tested at similar fish loading densities. In every case the sockeye were approximately half the size of the coho (due to stocks of fish on hand), hence size may be an influencing factor. GMST's for pink salmon that had just emerged from the gravel (30 mm, 0.2 g) were similar to 10-g coho in the same concentration, even though the fish density of pinks in solution was much lower.

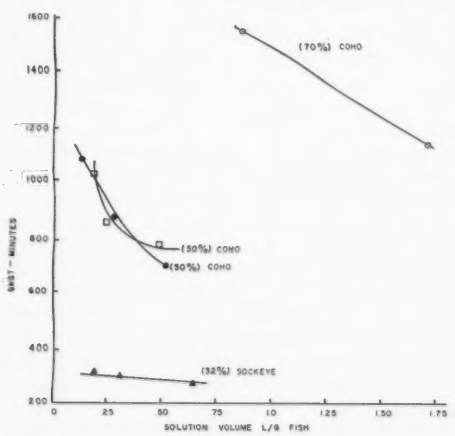

FIG. 3. The relation between fish density in the test solution and geometric mean survival time in full strength BKME. Each line represents a different effluent sample and its 4-day LC50 is indicated in brackets. Data are all for groups of coho and sockeye underyearlings.

Small fish seem quite resistant to BKME and Servizi et al. (1966) have observed that young sockeye salmon are more resistant to kraft mill bleach wastes than mature fish. In Servizi's tests, however, the adult fish were exposed for 2–4 weeks to renewed solutions and the juveniles to 96-hr tests. Thus the length of time of exposure may well have an influence on the results.

CONDITION FACTOR OF TEST FISH

To test whether size and general condition of bioassay fish influenced mortality within test groups condition factor was calculated for each fish according to Lagler (1956): $CF = 100\ (wt/leng^3)$ where wt = fish weight in g, leng = fish fork length in cm, and CF = condition factor. This value has been used as an index of the nutritional state and general health of a fish (Carlander 1950) and may be related to performance ability during exercise (Brett 1964). Condition factor was plotted against time to death for nearly 100 coho and 10 sockeye underyearlings in 12 bioassays with different full strength BKME samples. With the exception of one or two fish with very low condition factors there was no relation between size, weight, and susceptibility to BKME within the size range of fish tested. For coho this range was 0.24–4.05 g, 3.0–7.3 cm, and condition factors

TABLE 3. Examples of differing species sensitivity to BKME solutions in static bioassays. LC50's are for underyearling coho (1.5–15 g) at 10.5–12.5 ±0.5 C.

Effluent LC50 (% BKME)	Concn BKME %v/v	Liters soln./ g fish	Species	$\bar{X}$ wt(g)	No. killed	GMST (*min*)
55	100	.200	Coho	3.75	10/10	727
		.443	Sockeye	1.695	10/10	570
–	100	–	Coho	–	1/10	–
		.333	Sockeye	2.25	10/10	1619.5
50	50	.303	Coho	9.9	4/10	–
		.323	Sockeye	4.65	7/10	–
35	100	.467	Coho	10.7	6/6	374
		.308	Sockeye	4.87	10/10	285
		.649	Sockeye	4.56	10/10	267
		2.50	Pink	0.20	20/20	385
35	40	.467	Coho	10.5	6/6	1840
		.257	Sockeye	5.85	10/10	934
	55	.441	Coho	11.35	6/6	1120
		.263	Sockeye	5.7	10/10	557

ranged from 0.8–1.4 g/cm³. This size range seems acceptable provided that "weakling" fish with very low condition factors (<0.8) are omitted. Reimers (1963) reported that fish with condition factor in the range 0.6–0.7 "may be considered marginal in vitality, tiring quickly in stamina tests." Similar logic can probably be applied to toxicity tests.

EFFLUENT STORAGE

It has been reported that toxicity declines with storage, even at low temperature (Howard and Walden 1965; Servizi et al. 1966; Webb and Brett 1972; Werner 1963) and detoxification may be rapid when storage occurs at 16–21 C (Sprague and McLeese 1968a, b). Howard and Walden (1965) believed that detoxification during unaerated storage may be a chemical auto–oxidative process.

During 2 years experience with stored BKME we have observed the unpredictable nature of toxicity change with storage time. Table 4 summarizes these data. Some effluent samples declined in toxicity after periods of storage at 2 C (batches collected August 9, 1971; May 24 and June 30, 1972). Others indicated unchanged toxicity (September 20, 1971; January 31, 1972). Some increased (June 17, 1971; and full strength of June 30, 1972) in toxicity following storage.

NEW METHOD OF ANALYSIS

GMST was plotted against concentration of BKME for individual bioassays, and compared to the same plot using the median survival time estimated by graphical methods for LC50 determination. Comparison of the two (Fig. 1B) illustrates that for our data, GMST is close to, but consistently slightly higher than graphically estimated median survival time. Shepard (1955) found GMST to be the best statistical estimate of median response time for log-normal data.

When setting up 4-day LC50 tests it is helpful to know the appropriate BKME dilutions to be prepared to bracket the lethal threshold concentration. On the assumption that a rapid median survival time test could be used to predict the 4-day LC50, GMST's in full strength BKME were plotted against their corresponding LC50 value (Fig. 4). There is a good relation (Index of determination = .92) for the effluents we have tested. In our experience GMST of full strength BKME has consistently given a good estimate of LC50. A similar relation could be derived by plotting median survival times for 50% mortality (Fig. 1A) in full strength BKME against 4-day LC50 values. Use of GMST allows rapid mathematical manipulation of the data. This procedure could be useful for rapid, nonrigorous routine monitoring bioassays or for quickly checking stored effluent toxicity. It should be stressed that this result applied to the effluent, test fish, fish density, and conditions in our laboratory and may or may not apply in other situations. Regulatory or research *bioassays should of course determine the 4-day* LC50 in the conventional way (Sprague 1969).

TABLE 4. Toxicity of BKME to groups of underyearling salmon after various periods of effluent storage at 2 C. All groups are coho except as otherwise noted. LC50's were determined when effluent was initially collected.

Date batch collected	Days stored	Concn BKME %v/v	Liters soln./ g fish	No. killed	GMST (*min*)	Effluent LC50 (% BKME)
June 17, 1971	5	100	1.429	1/10	–	70
	12		1.000	10/10	1364	
Aug. 9, 1971	2	100	.200	10/10	727	55
	7		.200	1/10	–	
	2 (sockeye)		.445	10/10	570	
	7 (sockeye)		.333	10/10	1619	
Sept. 20, 1971	1	100	.526	10/10	956	65
	9		.476	10/10	933	
Jan. 31, 1972	1	100	.143	6/6	1066	57
			.278	6/6	827	
			.535	6/6	633	
	15	100	.173	6/6	942	
			.244	6/6	831	
			.486	6/6	758	
May 24, 1972	1	100	1.000	10/10	922	72
	7		1.000	7/10	–	
June 30, 1972	5	100	.588	10/10	210	22
	14	100	.556	10/10	143	
	5	50	.588	10/10	472	
	14	50	.556	9/10	–	
	5	30	.588	10/10	826	
	14	30	–	0/10	–	

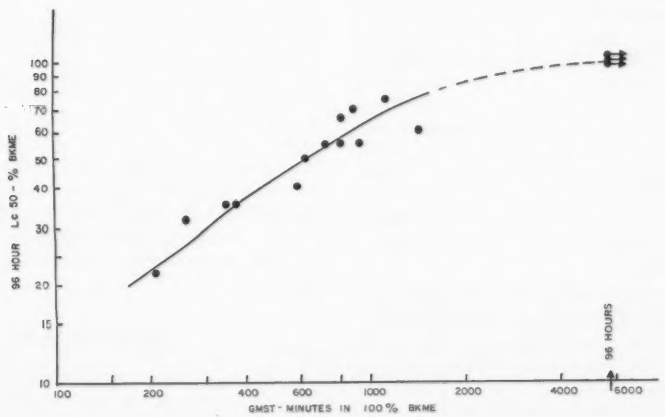

FIG. 4. The relation of the geometric mean survival time calculated from mortality in full strength BKME and the 4-day LC50 determined for a number of effluent samples. Data are for underyearling coho salmon, 0.8–14 g, in fresh water 20 ppm total hardness as $CaCO^3$, 11.1±0.2 C at a loading density of 0.56±.06 liter/g.

Discussion

In more than 2 years of testing the LC50 of bleached kraft pulp mill effluent from one mill varied from 22% to nontoxic at full strength, although most often it was 40–60%. This range of BKME toxicity is consistent with that reported for three British Columbia kraft mills (Howard and Walden 1965) but is considerably less toxic than that reported by Alderdice and Brett (1957) where the limiting concentration was 4.8% of full strength BKME in 20‰ salinity sea water. As discussed in Davis (1973) the lower toxicity found recently may relate to improved in-plant chemical recovery being practised by industry. BKME toxicity variability is complicated by the fact that detectable toxicity varies according to how the effluent is treated after collection, and how it is tested. The following discussion describes the sources of variable toxicity and how they may be standardized.

Airstripping of BKME prior to bioassay reduces measurable toxicity by removing volatile toxicants. However, due to varying amounts of these it is difficult to determine how much preaeration is necessary. The fact that most mortality in static BKME bioassays occurs in the first 48 hr suggests declining toxicity, possibly due to further airstripping. This problem can be overcome with continuous flow procedures (Sprague 1969). For investigative and regulatory purposes the decision must be made whether to do bioassays with air-stripped samples or attempt to deal with volatile toxicants and changing toxicity. One of the major deciding factors should be the environmental situation under consideration. If waste discharge occurs into large, well-mixed bodies of water, volatile toxicants will likely disappear rapidly in receiving waters. Under these conditions bioassay with air-stripped samples containing residual toxicants would seem reasonable. If however, the receiving waters are of limited capacity and turnover, the influence of volatile toxicants on acute toxicity should be included in the test procedure. On site bioassays with freshly discharged KME would seem the most reliable test in such circumstances.

Fish density is clearly one of the most important factors influencing measurable toxicity of BKME. It is only when solution volume exceeds 2.5 liters/g (Fig. 4) that density ceases to influence survival time. There is little published information on the relation of weight/volume ratios for bioassay tests with BKME. Comparisons are difficult as many workers report only the general size range of their fish. Most appear to have worked with 0.5–2.0 liters solution/g of fish (Alderdice and Brett 1957; Howard and Walden 1965, 1971; Sprague and McLeese 1968a, b; Servizi and Gordon 1973). More importance should be placed on this relation and be duly reported along with bioassay results. As the lower toxicity line in Fig. 3 indicates, a small change in wt/vol of static test solution near threshold toxicity could make a large difference in results. At least 2.5 liters/g solution should be used. This is in agreement with the 2–3 liters/g recommended by Sprague (1969).

Some differences in relative sensitivity of sockeye, coho, and pink salmon underyearlings to BKME were detected. Sockeye appeared most sensitive, and pinks and coho more resistant. These relationships were independent of fish size or fish weight/solution volume relations. Comparison of species is therefore difficult and wherever possible age, size, nutritional state, and test conditions (especially fish density) should be standardized if comparisons are to be made. New federal standards for toxicity testing of pulp mill effluent recommend rainbow trout as a standard test fish and define a size range for test fish. Guidelines of this type are needed for a variety of pollutants to be tested.

It is evident from our data that stored samples should be considered subject to changing toxicity and should be frequently checked. For regulative purposes the need for fresh samples, preferably at source, cannot be overemphasized. When on site testing is not practical, collection time and transport time should be minimized and samples should be cooled to low temperature and tested as soon as possible. As these studies indicate that toxicity may change in a nonpredictable fashion during storage, estimation of toxicity of fresh effluents from toxicity data for stored waste would seem risky.

The procedure described for estimating 96-hr LC50 from geometric mean survival time data in strong effluent solutions appears a useful tool that could possibly be applied to other toxicants. Although we have used geometric mean calculations to compute median survival time, graphical determination of this value could also be used to predict LC50. Experiments are planned to evaluate and test the applicability of this procedure with other toxicants.

Acknowledgments

We are indebted to Drs J. R. Brett, D. F. Alderdice, J. B. Sprague, J. A. Servizi, M. Waldichuk, and Mr T. E. Howard, all of whom reviewed the manuscript and offered helpful suggestions. The services of Mr I. Shand in collecting effluent samples and conducting bioassays are greatly appreciated. Mr C. Bulger and the laboratory staff of Canadian Forest Products, Howe Sound Pulp Division, were most cooperative in

effluent collections and in providing information on the operating conditions at the mill.

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NOTES

Mercury Uptake and Ion Distribution in Gills of Rainbow Trout (*Salmo gairdneri*): Tissue Scans with an Electron Microprobe

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OLSON, K. R., AND P. O. FROMM. 1973. Mercury uptake and ion distribution in gills of rainbow trout (*Salmo gairdneri*): tissue scans with an electron microprobe. J. Fish. Res. Board Can. 30: 1575–1578.

Mercury was found in gills of rainbow trout (*Salmo gairdneri*) which had been exposed to inorganic mercury but not in those exposed to methyl mercury. No specific site for mercury uptake was identified and it is suggested that inorganic mercury enters the gill across the general lamellar surface. High concentrations were found associated with the gill cartilage. Since little ion diffusion occurs during tissue preparation, localization and/or identification of tissues can be accomplished by scans for various elements: sodium (Na), potassium (K), chlorine (Cl), and sulfur (S). The technique is not suitable for identification of highly volatile compounds such as methyl mercury due to the necessity of subjecting tissues to high vacuum conditions, however, we believe electron probe analyses should be useful in studies of active ion transport systems in gill tissue and in investigations of the effects of heavy metal pollutants on fishes.

OLSON, K. R., AND P. O. FROMM. 1973. Mercury uptake and ion distribution in gills of rainbow trout (*Salmo gairdneri*): tissue scans with an electron microprobe. J. Fish. Res. Board Can. 30: 1575–1578.

Nous avons trouvé du mercure dans les branchies de truites arc-en-ciel (*Salmo gairdneri*) qui avaient été exposées au mercure inorganique, mais non dans celles exposées au mercure-méthyl. Nous n'avons pu localiser de site particulier d'absorption du mercure et suggérons qu'il pénètre dans les branchies à travers la surface lamellaire entière. Le cartilage branchial en contient de fortes concentrations. Puisqu'il y a peu de diffusion ionique pendant la préparation des tissus, la localisation et/ou l'identification des tissus peut se faire par balayages visant divers éléments: sodium (Na), potassium (K), chlorine (Cl), and sulfur (S). La méthode ne convient pas pour l'identification de composés hautement volatils, tel le mercure-méthyl, à cause de la nécessité de soumettre les tissus à un vide poussé; cependant, à notre avis, des analyses à la sonde électronique devraient être utiles pour des études du transport ionique actif dans le tissu branchial et pour des recherches sur les effets des métaux lourds polluants sur les poissons.

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GILLS of freshwater fishes, including the rainbow trout, are bathed by large volumes of ambient water. Passive loss of ions such as sodium and chloride from the fish can be made up by their active inward secretion across the gill. In an investigation of the effect of mercury on gills of fishes we obtained information on the ultrastructural characteristics of trout gills (Olson and Fromm 1973) and on the efficiency of the gills as uptake sites for organic and inorganic mercurials (Olson et al. 1973).

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Efforts to localize and/or characterize the pathway of uptake of mercury in gills of trout using radioautographic techniques were largely unsuccessful. Autographs of gills of fish exposed to either methyl or inorganic mercury exhibited a diffuse grain pattern which precluded identification of the uptake site. Lack of grain density in areas containing many chloride cells probably indicates that these cells are not involved in the uptake of either mercurial. The thinness of lamellae and the small interlamellar distances prevented localization of preferential mercury binding sites. Dense grain patterns due to $^{203}Hg^{++}$ were found associated

with the filamental cartilage presumably associated with the chondroitin sulfate ground substance of the cartilage (Leeson and Leeson 1970).

The use of radiolabeled compounds has shown that methyl and inorganic mercury is taken up by trout via the gills (Olson et al. 1973). Our second attempt to localize the gill pathway for mercury uptake was done with the electron microprobe. The following is a report of these experiments.

Methods — Rainbow trout (*Salmo gairdneri*) weighing 50–70 g were obtained from the Michigan Department of Natural Resources hatchery at Grayling, Mich. In the laboratory they were kept in fiberglass tanks which were continuously flushed with water at 13 C.

Fish were exposed to the mercurials which were added to water in 100-liter tanks. The concentrations used were 0.25 and 0.05 ppm Hg for inorganic ($HgCl_2$) and organic (CH_3HgCl) mercury, respectively. After 23-hr exposure a second amount of mercury equal to the first was added to the tanks and fish were removed 1 hr later and killed.

The second pair of gill arches, removed from mercury-exposed fish within 15 sec after the fish was killed, was quick frozen in isopentane (2 methyl butane, Eastman Kodak Co.) which had been cooled to near –160 C with liquid nitrogen. Frozen tissues were then mounted on a brass stub and embedded with Ames O.C.T. compound at –30 C (Ames Company Division, Miles Labs. Inc., Elkhart, Ind.). Frozen sections 4–10 μ thick were cut with a microtome and fastened with Eastman 910 adhesive to either quartz slides or carbon discs. After the 910 adhesive had polymerized (1–3 hours at –30 C) the tissues were freeze-dried for 36 hr then stored in a desiccator until just prior to examination. No more than 12 hr elapsed between completion of freeze-drying and initiation of the microprobe studies in order to reduce movement of ions due to tissue rehydration. Small crystals of $HgCl_2$ were glued to a corner of each slide and were at least 5 mm from the tissues. These crystals were used to determine the optimal settings for the spectrometer at the start of each experiment.

Dried tissues were carbon coated in a Varian model VE10 evaporator and placed in an ARL Microprobe, Model EMX-SM (Applied Research Laboratories, Sunland, Calif.) for examination. Accelerating electron voltages ranged up to 30 KV with the elements in question and were generally in excess of 23 KV for mercury analyses. Elemental analyses were accomplished by tissue scans for characteristic X-ray emission. These were then compared to an image scan of the tissue (secondary electron detection which produces the standard scanning electron micrograph, SEM) or to a single line scan with X-ray activity plotted on an x-y recorder as a function of distance across the tissue. The tissue scan and image scan micrographs were recorded on Polaroid 200 black-and-white film and the line scan was recorded on a Hewlett Packard x-y recorder. Low peak-to-background ratios during mercury analysis made the tissue scan procedure difficult to interpret, however, line scan procedures proved adequate for mercury identification. Line and tissue scans were also used for detection of other elements as both an aid in tissue orientation and a check on diffusional ion movements which might have occurred during sample preparation. The 0.5-μ diameter of the primary electron beam permits fairly high resolution in the localization of ions within a given area especially with the line scan procedure. All analytical data given are qualitative or only semiquantitative as the refinements and sophistication required for quantitative determinations are beyond the scope of this preliminary study.

Results and discussion — Using the microprobe we were unable to detect mercury in gills from trout exposed to methyl mercury even though previous studies (Olson et al. 1973) showed that in trout exposed to $CH_3{}^{203}HgCl$ the gills contained 10 times more label than those from $^{203}HgCl_2$-exposed fish. The inability to detect mercury after methyl mercury exposure was probably due to loss of this element during tissue preparation. Crystals of methyl mercury will sublime at room temperature, therefore, mercury could be lost during freeze drying, carbon evaporation, or in the vacuum column of the microprobe. All of these processes require a high (around 5 millitorr) vacuum.

Figure 1 shows the secondary electron output (SEM image) from a cross section through a filament and several lamellae. The horizontal line depicts the beam tracing which corresponds to the line scan in Fig. 2. From left to right the horizontal line in Fig. 1 traverses the filamental sinus (Richards and Fromm 1969), enters filamental tissue and passes through the basal area of a lamella where chloride cells are localized (see arrow). The line then crosses three lamellae (white areas) and associated interlamellar spaces.

The line scan (Fig. 2) shows the relative distribution of Na, K, Ca, and Cl and their relation to mercury. The abscissa is divided into segments which correspond to the large squares in Fig. 1 (10 squares full scale). The ordinate corresponds to the relative intensity of X-radiation emitted by each element. Quantitative comparisons of elements are prevented by the use of different sensitivities for each element and displacement of the base line for graphic display. Recordings on the left edge of Fig. 2, between the ordinate and the first division, correspond to a blood vessel or sinus space which is off the field of view in Fig. 1.

Peaks in the tracings for sodium and chloride at the far left in Fig. 2 indicate a tissue blood space. In subsequent observations it was found that sodium is not an ideal plasma marker because in the vascular space it becomes volatile when subjected to an electron beam and can be lost from the tissue. Why large quantities of chloride, the most abundant

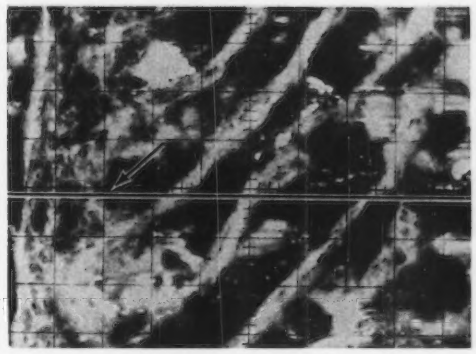

FIG. 1. Scanning electron micrograph of a cross section of a trout gill filament. The solid horizontal line corresponds to the beam pathway which corresponds to the line scan shown in Fig. 2. For details see text.

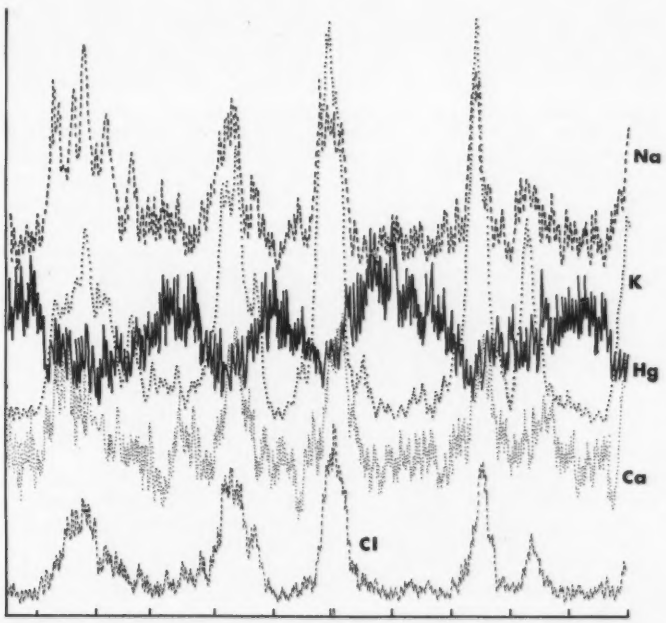

FIG. 2. Line scan of tissue shown in Fig. 1. Sodium (Na), potassium (K), mercury (Hg), calcium (Ca), chlorine (Cl). Beam path for scan corresponds to horizontal line in Fig. 1. Abscissa divisions correspond to grid divisions along beam path in Fig. 1. Ordinate has no units — see text.

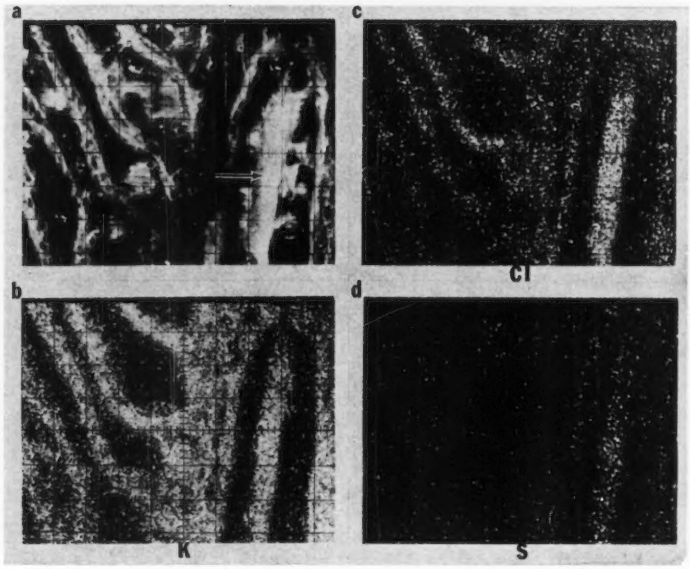

FIG. 3. Tissue scan of lon itudinal cross section through the body of a filament and lamellae. Figure a, SEM image (400X). Figure b, c, and d, elemental scans for potassium (K), chlorine (Cl), and sulfur (S), respectively.

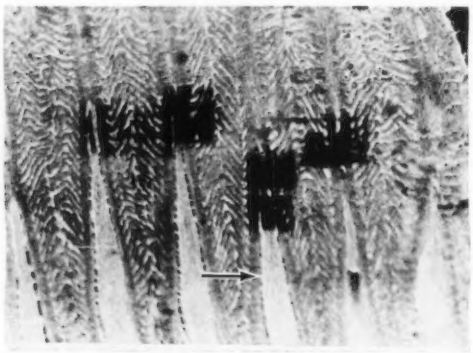

FIG. 4. Longitudinal section of gill filaments. Arrow points to filamental cartilage. The darkened areas indicate the areas scanned by the electron microprobe during a routine experiment.

anion in the plasma, were not found in the vascular space is unknown. Some could have been lost by diffusion although this is unlikely since chloride peaks correspond well to the location of lamellae. The strong mercury peak at the far left of Fig. 2 suggests that the paucity of other ions in this area does not reflect tissue separation. The relatively high levels of mercury found in blood following exposure to $HgCl_2$ could produce a mercury peak similar to that which was observed.

As the beam entered the area known to contain large numbers of chloride cells, concentrations of all ions except mercury increased. Interlamellar areas contained definite mercury peaks and the location of lamellae was marked by increases in the concentrations of Na, K, Ca, and Cl (Fig. 2). Mercury content appeared to be inversely related to concentrations of the other elements and it did not increase until the beam entered the space normally filled by environmental water. This confirms results of radioautographic studies and supports the concept that inorganic mercury is not selectively taken up by chloride cells nor concentrated by them in 24 hr. Other line scans of areas of high chloride cell density on both horizontal and vertical axes substantiated this finding. The mercury peaks are due in large part to mercury which was added 1 hr prior to sampling and demonstrated the effectiveness of the interlamellar mucus to trap ions. Presumably mercury is contained in the mucus from which it can either enter the gill or be eliminated with the sloughed off mucus. McKone et al. (1971) exposed goldfish to 0.25 ppm $HgCl_2$ for 3 hr and found that 79.3% of the mercury in excised gills was removed by washing with 80% ethanol. They concluded that this fraction represented mercury trapped by the mucus coating the gill, however, they did not examine gill tissues after the ethanol wash to verify that only mucus was removed. Gills are very fragile and it is quite possible that some epithelium was also removed in the wash process.

Figure 3a is an SEM micrograph of a longitudinal section through a filament. Cartilage is indicated by an arrow and lamellae, extending toward the upper right, can be seen leaving the filament in the center of the micrograph. Elemental scans are as indicated in Fig. 3b, c, and d. Clarity of the lamellae in the elemental scans is indicative of the low degree of ion diffusion during tissue preparation.

Tissue scans of other material showed that mercury concentrations were highest in cartilage and were correlated with high calcium, sulfur, and phosphorus levels. Microprobe (and radioautographic) studies have clearly indicated that there is no preferential accumulation of mercury in lamellae. It appears that mercury can enter gills through the general lamellar surface, thus active uptake is probably of little consequence in mercury influx. Once in the filament, much inorganic mercury is accumulated by cartilagenous tissue. Other gill tissues exhibit no preferential uptake of mercury with respect to a given cell type, i.e. epithelial cells, pillar cells, or chloride cells.

The electron microprobe has been used extensively in analyses of a variety of biological specimens (Hall et al. 1966; Tousimis 1962, 1963). To our knowledge this is the first demonstration of the use of this technique for identification of uptake sites for mercury or the distribution of ions in gills of fishes. Some indication of the size of tissue sample which can be examined with the microprobe is given in Fig. 4 which is a light micrograph of a longitudinal section of six filaments (40X). The black squares are areas examined by the microprobe during a routine experiment. The arrow points to the filamental cartilage which has been sectioned obliquely.

Preparation of tissues for electron microprobe analysis avoids the use of various aqueous solvents as are commonly employed in routine analytical histochemistry. Thus, localization of many ions (e.g. small molecular weight monovalents) with the probe probably reflects a fairly true tissue distribution of these ions. We suggest that electron probe analyses should prove beneficial in a study of ion distribution in chloride cells of fish exposed to various salinities or to pharmacological agents. Further information on the ion transfer role of mucus, which covers the surface of chloride cells from freshwater fish or that present in the apical crypts of cells from marine species, could be gained by studies utilizing the electron microprobe.

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First Record of Bacterial Kidney Disease in an Apparently Wild Salmonid in British Columbia

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EVELYN, T. P. T., G. E. HOSKINS, AND G. R. BELL. 1973. First record of bacterial kidney disease in an apparently wild salmonid in British Columbia. J. Fish. Res. Board Can. 30: 1578–1580.

A confirmed case of bacterial kidney disease in a sports-caught rainbow trout (*Salmo gairdneri*) from Loon Lake, British Columbia, is reported. The case represents the first record of this disease in an apparently wild fish in this province. Descriptions of the affected trout and of the causative bacterium are provided.

EVELYN, T. P. T., G. E. HOSKINS, AND G. R. BELL. 1973. First record of bacterial kidney disease in an apparently wild salmonid in British Columbia. J. Fish. Res. Board Can. 30: 1578–1580.

Nous signalons un cas authentique de maladie bactérienne du rein chez une truite arc-en-ciel (*Salmo gairdneri*) capturée à la ligne dans le lac Loon, Colombie-Britannique. C'est la première mention de cette maladie chez un poisson apparemment sauvage de cette province. Nous décrivons l'individu affecté et la bactérie pathogène.

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BACTERIAL kidney or Dee disease was probably first described in the 1930s in wild Atlantic salmon (*Salmo salar*) in Scotland (work cited by Smith 1964) and in three species of cultured trout in the United States (Belding and Merill 1935). It seems likely that early workers were indeed dealing with the kidney disease (KD) bacterium because the organism was a small, gram-positive bacillus which evidently could not be cultured.

Although the KD bacterium can now be grown with some difficulty on artificial media (e.g. Ordal and Earp 1956), diagnosis of bacterial KD seldom involves isolation of the causative bacterium and workers are usually content to diagnose infections presumptively. In practice, presumptive diagnosis is based largely on demonstrating the presence (usually in the kidney) of a small, gram-positive diplobacillus in the tissues. A requirement that the bacterium also be shown to be nonacid-fast (Bullock 1961), is seldom met.

Infections with the KD bacterium have been presumptively diagnosed in wild salmonids in Canada (Pippy 1969) and the United States (MacLean and Yoder 1970). Only once, however, has a confirmed diagnosis, involving isolation of the bacterium, been reported in wild fish in North America (Wood and Wallis 1955).

The purpose of this report is to record for the first time a confirmed case of bacterial KD in an apparently wild salmonid in British Columbia.

The specimen — The infected fish was a female rainbow trout (*Salmo gairdneri*) that had been caught by angling in mid-September, 1971, in Loon Lake, B.C. (For a description of Loon Lake and its location, see Lindsey et al. 1959.) The fish had been retained because

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of a 1 cm^2 area of reddened tissue that underlay apparently intact skin in the ventro-pelvic region and had been kept frozen until its receipt at this laboratory on November 17, 1971. The specimen weighed 312 g, measured 31 cm in fork length and, based on egg-size (2.0–2.5 mm in diameter), would likely have spawned in the following spawning season.

As characteristic of wild trout in Loon Lake, the fish was brightly pigmented externally and its flesh was a striking orange-pink. The fish had recently been feeding and the only gross abnormalities found were the previously-mentioned area of reddening, which proved to be superficial, and a small lesion in the ventral aspect of the body wall anterior to, and not visibly connected to, the reddened area. When the lesion was ruptured, a white turbid fluid was released and a cavity of about 0.5-ml capacity, lined with necrotic muscle, was revealed. The exudate was composed of disintegrating white blood cells, muscle fiber fragments, and very low numbers of small, gram-positive bacilli. Single and paired bacilli usually occurred as widely scattered units in gram-stained smears of the exudate. No bacteria were observed in gram-stained smears of kidney and spleen.

The bacterium — When purulent material was cultured at 15 C, growth resulted only when specialized media devised for growing the KD bacterium were used. There was no growth on a tryptone-yeast extract agar when plates of this medium, wrapped in plastic film, were incubated for 12 weeks. This medium was known to be incapable of growing the KD bacterium but it was routinely used in this laboratory to screen fish for the presence of less fastidious fish pathogens (Evelyn 1971a, b). However, on media prepared with human plasma (in place of whole blood) such as Ordal and Earp's (1956) KD medium (KDM1) and a local modification of this medium (KDM2), well-isolated colonies of an apparently pure bacterial culture were obtained.

The isolate grew slowly like known (hatchery) strains of the KD bacterium used for comparison. As was invariably true for the known strains, growth of the isolate appeared first on KDM2 and otherwise was indistinguishable from that obtained on KDM1. On KDM2 the colonies were just visible at 26 days, and it was 35 days before they measured 1 mm in diameter. From the distribution and paucity of bacterial cells in gram-stained smears of the exudate, it seems reasonable to conclude that the colonies were derived from single cells. Assuming this to be true, the observed growth rate was very similar to that obtained with known strains of the KD bacterium, uniform cell suspensions of which had been diluted to the stage where the colonies developed from single cells.

Morphologically and culturally, the isolate was identical with the reference strains of the KD bacterium. Cells, grown on KDM2, resembled those seen in smears of the exudate; they were short rods that averaged 0.5 × 0.9 μm when gram-stained; they were gram-positive, nonacid-fast, noncapsulate, and nonspore-forming, and occurred singly and in pairs. Like the reference strains, cells were nonmotile. Discrete, month-old colonies on KDM2 plates were creamy-white, circular, convex, entire, and moderately shiny. On further aging, the colonies changed in consistency from butyraceous to sticky. On uncrowded plates (5–20 colonies per plate), a maximum colony-size of about 2.5 mm in diameter was attained in about 2 months. However, on crowded plates, colonies were usually less than 1 mm in diameter and they conferred the "ground-glass" effect to the agar surface (Smith 1964). With inocula heavy enough to cause crowding, growth appeared to be accelerated and was maximum at about 2 weeks. In broth, such as KDM2 prepared without agar, the medium never became cloudy. Growth occurred initially as a powdery sediment, but after a month or so, a pellicle was produced. Typically also, the isolate behaved like an aerobe. In tubes of uniformly inoculated sloppy agar (KDM2 prepared with 0.075% agar), growth at 3 months was still only evident in the top third of the medium. The isolate also resembled the KD bacterium in that it grew best at about 15 C, failed to grow at 25 C, required cysteine for growth, and with dilute inocula, grew only when a supplement such as human plasma was present in the medium.

As characteristic of our strains of the KD bacterium, dense cell suspensions (a calculated 10.0 OD at 420 nm) of the isolate in saline underwent a rapid auto-agglutination that was effectively prevented when the suspensions were heated for 45 min at 62 C to kill the cells. Heated cells of the isolate were serologically similar to those of known hatchery strains of the KD bacterium. When they were reacted with sockeye salmon (*Oncorhynchus nerka*) serum that had been shown to be specific for the KD bacterium (Evelyn 1971c), they were agglutinated to the same extent (titer 1: 10,240) as those of two tested strains of the KD bacterium, one of which had been used to prepare the antiserum.

Pathogenicity of the isolate was confirmed using sockeye salmon averaging 86 g held in fresh water at 13–14 C. Four fish were each injected intraperitoneally with 0.2 ml of saline-washed, 17-day-old, KDM2 grown cells suspended in 0.1% peptone–0.9% NaCl (PS) to an opacity of 1.0 or 10.0 OD at 420 nm. These fish died in 22 to 36 days, and in 17 to 31 days, respectively. The resulting "times-to-death" and disease signs were all characteristic of bacterial KD, and were essentially identical with the results obtained in a concurrent experiment in which a freshly-isolated hatchery strain of the KD bacterium was used. In all instances, cells resembling those of the injected organism were seen, often phagocytized, in gram-stained smears of kidney material, and the organism was re-isolated in pure culture from the kidneys of fish examined by cultural methods. Control fish that received appropriate amounts of PS, or of heat-killed cells in PS, all survived. The experiment was terminated at 52 days postinoculation and, on necropsy, the kidneys of control fish proved to be free of bacteria by microscopic and cultural methods and, the fish appeared to be grossly normal.

Discussion — Based on the characteristics of the isolate, there can be little doubt that the rainbow trout was infected with the KD bacterium. This bacterium has for many years caused problems in

stocks of cultured Pacific salmon held at three west coast facilities of the Fisheries Research Board of Canada (e.g. Bell 1961). Its presence, however, in the Loon Lake rainbow trout constitutes the first direct evidence that the bacterium also occurs in fish in natural waters in British Columbia.

The present finding is regarded as most likely representing a natural case of bacterial KD because the Loon Lake system has always depended on natural reproduction for the maintenance of its sizeable rainbow trout population. (In 1971, Loon Lake produced about 32,000 spawners and an estimated angler catch of 60,000 fish (B.C. Department of Recreation and Conservation Annual Report for 1971).) However, Loon Lake is used as a point from which hatchery-reared trout are distributed by float-plane to other lakes in the region. During loading of the float-plane, the occasional hatchery fish has escaped to Loon Lake. (Fish for stocking are reared at, or are brought into, a small, seasonally-operated fish-holding facility located on the outlet stream from Loon Lake below rapids impassable to the upstream movement of fish.) While bacterial KD has never been diagnosed in any of the B.C. trout hatcheries involved in lake stocking programs, the possible role of the hatchery escapee in explaining the present case of bacterial KD cannot be entirely ruled out.

Twelve additional rainbow trout from Loon Lake have been examined. Of these, 10 were sexually mature fish that were collected in the lake by gillnetting on December 17, 1971. Two others, collected on the spawning beds on April 23, 1972, were ripe but moribund. In none of these fish was there any evidence (gross, microscopic, or cultural) of infectious disease. In three of the fish, the hearts were quite heavily encrusted with cysts of a larval trematode (*Tetracotyle* sp.), which, in the case of the two trout collected on the spawning beds might, conceivably, have accounted for their sluggish behavior.

Because of the small number of fish examined from Loon Lake, it is impossible to draw conclusions as to the nature and severity of the diseases affecting rainbow trout in the lake. It appears, however, that the operation of a fish-holding facility in a water supply subject to contamination with the KD bacterium, should be reviewed.

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Apparatus for Simulating Motion of Animals through the Geomagnetic Field

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ROMMEL, S. A. JR. 1973. Apparatus for simulating motion of animals through the geomagnetic field. J. Fish. Res. Board Can. 30: 1581–1582.

An apparatus to simulate animal motion through the geomagnetic field was devised. The test animal can be rotated through a magnetic field produced by two coils of wire. With this equipment biologists may introduce motionally induced electric fields into laboratory experiments on electro- and magneto-sensitivity.

ROMMEL, S. A. JR. 1973. Apparatus for simulating motion of animals through the geomagnetic field. J. Fish. Res. Board Can. 30: 1581–1582.

On a inventé un appareil pouvant imiter le mouvement d'un animal dans un champs géomagnétique. L'animal soumis aux essais peut être tourné dans un champs magnétique produit par deux bobines de fils. Cet appareil permet aux biologistes d'introduire des champs électriques induits par mouvements dans des essais de laboratoire sur la sensibilité à des champs électriques et magnétiques.

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IN most experiments which have shown a magneto-sensitivity the animals have had some motion relative to the test magnetic field (e.g. Brown 1962, 1971; Gleizer and Khodorkovsky 1971; Keeton 1971; Wiltschko and Wiltschko 1972). Because of this motion it is difficult to separate magnetic effects from effects of electric fields generated by movements of the animals in relation to these magnetic fields.

The total electric field intensity is described by:

$$\overline{E} = \overline{U} \times \overline{B} - \rho\overline{J}$$

where $\overline{U} \times \overline{B}$ is the electric field intensity generated by charged particles as they move at velocity $\overline{U}$ in the presence of the magnetic field, $\overline{B}$; $\rho\overline{J}$ is the electric field intensity due to current density, $\overline{J}$, moving through a medium of resistivity, ρ. Experiments with Atlantic salmon (*Salmo salar*) and American eels (*Anguilla rostrata*) (Rommel and McCleave 1972, 1973a) have shown that these two fishes can be conditioned to a weak $\rho\overline{J}$ field; no such results were obtained in testing their response to a stationary magnetic field ($\overline{U} \times \overline{B}$ where $\overline{U} = 0, \overline{B} \neq 0$). The experimental facilities were inadequate to determine whether these fishes respond to electric fields induced by motion through a magnetic field ($\overline{U} \times \overline{B}$ where $\overline{U} \neq 0, \overline{B} \neq 0$).

This report describes an apparatus that can move animals through a test magnetic field and therefore introduce the $\overline{U} \times \overline{B}$ term (motionally induced electric fields) where neither $\overline{U}$ nor $\overline{B}$ is equal to 0.

Apparatus — *Two subcylindrical plexiglass tanks (16* cm wide × 30 cm high × 100 cm long) are mounted on a wheeled platform and driven by an electric motor (Fig. 1). This arrangement rotates the tanks in a circle 1 m in radius (to center of tank) at a linear velocity of 1 m/sec.

The magnetic field is produced by two horizontal coils of wire 270 cm in diameter, spaced 64 cm apart vertically. Coil diameter was limited by room size. The vertical spacing optimizes magnetic field uniformity for the test volume (a cylinder 4 cm high × 8 cm wide, centered at a radius of 1 m) through which the tanks are moved. An electric current of 0.5 amp produces a magnetic field of 0.5 gauss in the test region.

When the electric currents in the two coils are flowing in the same direction the net magnetic field within the test region is vertical; the horizontal components of the two coils in the test region cancel each other. When the electric currents are opposite in the two coils the net magnetic field is horizontal; the vertical components cancel each other. Theoretical field uniformity within the test volume is better than ± 1%. Laboratory measurements with a Hall effect gaussmeter (± 3%) showed no deviation from the predicted field.

Electric signals (e.g. for taking a fish electrocardiogram) can be led to or from the tanks via leads through a commutator at the center of the rotation apparatus. The commutator consists of four aluminum conduit

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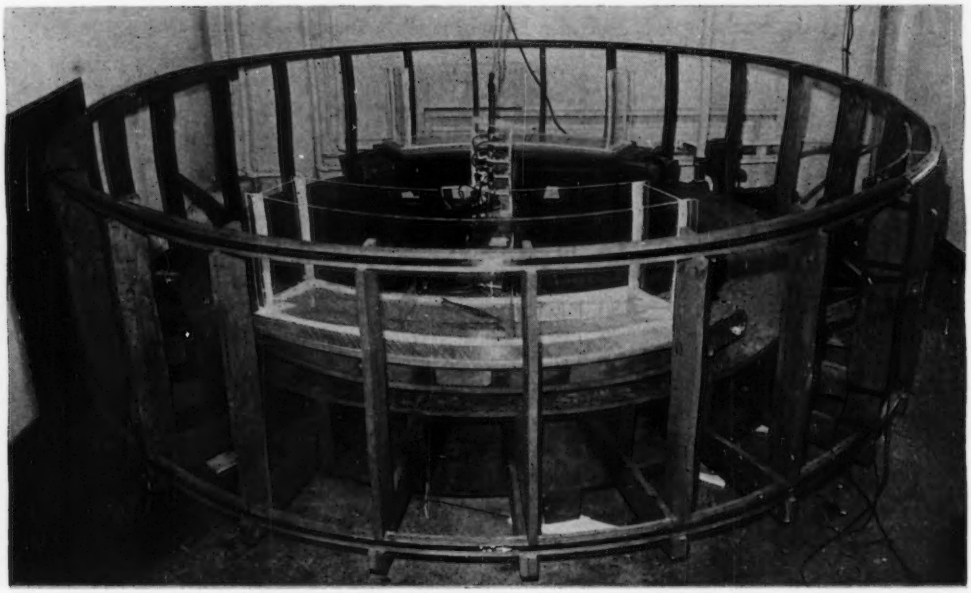

FIG. 1. The apparatus. The two plexiglass tanks are moved by the electric motor in the center. The magnetic field is produced by the two horizontal coils; 270 cm diameter and separated vertically by 64 cm.

collars threaded on a PVC pipe and rotating carbon brushes held in place by a plexiglass holder.

To date only salmon have been placed in the apparatus. They appear to overcome initial reactions to the tank motion within a few minutes.

Discussion — The conditioning technique used by Rommel and McCleave (1972, 1973) eliminates the effects associated with extraneous electric fields since the fish is conditioned only to field changes associated with changes in the test magnetic field. This is also true for visual and vestibular effects associated with motion within the apparatus.

The usefulness of the apparatus is not limited to study of aquatic organisms. Any animal moving through the geomagnetic field has induced some electric fields within its body. Replacement of the tanks with appropriate holding cages would make the equipment usable by other orientation investigators.

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Gross Structure of the Adductor Muscles of Some Pelecypods

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MORRISON, C. M., AND P. H. ODENSE. 1973. Gross structure of the adductor muscles of some pelecypods. J. Fish. Res. Board Can. 30: 1583–1585.

A study of the gross structure of adductor muscles of the following pelecypods showed that they conform to Morton's grouping into the a) "Protobranchia" (*Nucula proxima* and *Yoldia limatula*), b) "shallow-burrowing lamellibranchs" (*Clinocardium ciliatum, Venericardia borealis, Astarte undata, Arctica islandica, Venus mercenaria*, and *Spisula solidissima*), c) "surface attached lamellibranchs" (*Mytilus edulis, Modiolus modiolus, Modiolus demissus, Placopecten magellanicus*, and *Crassostrea virginica*), d) "deep-burrowing and immobile lamellibranchs" (*Ensis directus, Hiatella arctica*, and *Mya arenaria*); thus providing more evidence for his classification. The adductor muscle is divided into two portions — translucent and opaque — except in the "deep-burrowing and immobile lamellibranchs", which have opaque muscles only.

MORRISON, C. M., AND P. H. ODENSE. 1973. Gross structure of the adductor muscles of of some pelecypods. J. Fish. Res. Board Can. 30: 1583–1585.

Une étude de la structure macroscopique des mus les adducteurs des pélécypodes suivants démontre qu'ils sont en accord avec le groupement qu'en fait Morton en a) "Protobranchia" (*Nucula proxima* et *Yoldia limafula*), b) "lamellibranches fouisseurs peu profonds" (*Clinocardium ciliatum, Venericardia boreaţis, Astarte undata, Arctica islandica, Venus mercenaria* et *Spisula solidissima*), c) "lamellibranches fixés sur une surface" (*Mytilus edulis, Modiolus modiolus, Modiolus demissus, Placopecten magellanicus* et *Crassostea virginica*), d) "lamellibranches fouisseurs profonds et immobiles" (*Ensis directus, Hiatella arctica* et *Mya arenaria*), fournissant une preuve de plus à l'appui de sa classification. Le muscle adducteur comprend deux portions — transłucide et opaque — sauf chez les "lamellibranches fouisseurs profonds et immobiles", qui n'ont que des muscles opaques.

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MANY attempts have been made to classify the pelecypods. In one classification, the Pectinidae, Mytilidae, and Ostreidae are placed with the Nuculidae and Nuculanidae in the primitive order Priodesmacea (Miner 1950). Usually, however, only the Nuculidae, Nuculanidae, and Solemyidae are placed in the primitive order Protobranchia (Barnes 1963; Morton 1968). Often the pelecypods are classified by gill structure, into Protobranchia, Filibranchia, Eulamellibranchia, and Septibranchia (Barnes 1963), thereby inferring that the complexity of gill structure increases with this species' evolution. However, the use of one character is not satisfactory, since parallel evolution may have occurred. Classification may be more satisfactory if other structural and ecological characters are included. One such classification divides the pelecypods into the Protobranchia — a separate primitive group, first in order of appearance in the fossil record — and "shallow-burrowing", "surface attached", and "deep-burrowing and immobile" lamellibranchs — (Morton 1968).

We investigated the gross structure of the adductor muscles of several pelecypods, in search of another character to aid classification.

Materials and methods — The pelecypods investigated are listed in Table 1. One valve of each living specimen was removed by severing the hinge ligament and the insertion of the adductor. The preparations were photographed with an Edixa camera, using black-and-white 35 mm Panatomic X film. Prints were made on Kodak F5 Kodabromide paper.

The adductors of *N. proxima* and *Y. limatula* are too small to determine by gross inspection whether they are

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divided into two parts. The adductor of *N. proxima* was therefore fixed for light microscopy. The valves were wedged open, and the preparation was fixed in Zenker–acetic acid fixative (Humason 1962). The valves were removed and the specimen was washed, dehydrated, cleared, and embedded in paraffin. Sections 7μ thick were stained with hematoxylin and eosin.

Results — In *N. proxima* and *Y. limatula*, the anterior and posterior adductors are almost the same size (Fig. 1A, B). The respective anterior and posterior adductors of *S. solidissima* (Fig. 2A), *A. islandica* (Fig. 2B), *V. mercenaria* (Fig. 2C), *A. undata* (Fig. 2D), *V. borealis* (Fig. 2E), and *C. ciliatum* (Fig. 2F), which also are nearly equal in size, are divided into a translucent portion usually tinted pink, and an opaque white portion. The peripheral parts of the adductor are opaque and the internal parts next to the viscera are translucent. The proportion of translucent to opaque muscle is nearly equal in the anterior and posterior adductors. In *A. islandica*, *V. mercenaria*, and *C. ciliatum*, the translucent part of the adductor muscle is almost the same size as the opaque part; in *S. solidissima*, the translucent part is larger, and in *A. undata* and *V. borealis* the opaque part is greater.

Modiolus modiolus (Fig. 3A), *M. demissus*, and *M. edulis* have asymmetrical anterior and posterior adductors, divided into opaque and translucent portions, the translucent part being slightly larger than the opaque part; the posterior adductor is the larger in both species. *Placopecten magellanicus* and *C. virginica* have only one adductor (Fig. 3B, C), whose translucent part, especially in *P. magellanicus*, is larger than the opaque. After the organism's death, the translucent portion becomes more opaque than was the opaque part in life.

Ensis directus (Fig. 4A), *M. arenaria* (Fig. 4B), and *H. arctica* (Fig. 4C) have anterior and posterior adductors of different shapes. The anterior appears to be the larger adductor in *E. directus*, but the posterior one is the larger in *M. arenaria* and *H. arctica*. In *E. directus* the anterior adductor is elongated along the main axis of the valves and the posterior one is small and round. *Mya arenaria* and *H. arctica* also have slightly elongated anterior adductors parallel to the valves, and compact, round posterior adductors. The fibers of the adductor muscles of *N. proxima* (Fig. 5) are at the broad end of each adductor.

Discussion — *Nucula* was earlier reported to have an adductor with two types of muscle (Grassé 1960; Bowden 1958), and light microscopy has confirmed this for *N. proxima*. These features may reflect a lack of specialization of protobranchs and shallow-burrowing lamellibranchs compared with other pelecypods, since both groups burrow more slowly into soft mud or sand than do some of the more elongated pelecypods. The anterior and posterior adductors are used for burrowing (Trueman 1968). The shell is moderately symmetrical laterally and anteroposteriorly, and the shape of the adductors reflects this symmetry. According to various authors (e.g. Bowden 1958), the translucent muscle is usually striated or obliquely striated, whereas the opaque muscle is usually smooth and is slower than other types. The fast and slow muscles probably are used in burrowing. The fast muscle can contract briefly to clear the mantle cavity of detritus. It can close the valves rapidly in adverse conditions — for instance, when water temperatures are abnormal or when predators or noxious substances are present — and can keep the valves closed if adverse conditions persist. In *A. islandica* the translucent muscle is obliquely striated and the adductor muscle smooth and opaque (Morrison and Odense 1973). Other authors (Bowden 1958) have found the same in *Cardium* (related to *Clinocardium*) and *Venus*. In these three pelecypods the proportions of translucent and opaque parts of the adductor are almost equal. In *A. undata* the translucent muscle is striated (Morrison and Odense 1973), and is smaller than the opaque part. The faster contraction rate of striated than obliquely striated muscle may compensate for the smaller mass of translucent muscle.

The anterior adductor is smaller or absent in surface attached lamellibranchs. The Mytilidae are oriented vertically on the substrate and have laterally symmetrical valves, whereas the Ostreidae and Pectinidae have lost this symmetry and lie on the flattened right valve. This difference is paralleled by modifications in the form of adductors. The Mytilidae (*M. modiolus*, *M. demissa*, and *M. edulis*) retain two adductors, although the anterior adductor is small; however, *C. virginica*, a member of the Ostreidae, and *P. magellanicus*, a Pectinidae have only one adductor. The habitat of *M. modiolus* suggests that this mussel has some burrowing capacity, requiring two adductors. Since many Mytilidae, Ostreidae, and Pectinidae do not burrow and are more specialized for a sessile existence, two adductors are apparently unnecessary and reduction has occurred. The presence of only one adductor may be an advantage to the Ostreidae and Pectinidae, because it can be large and central, facilitating rapid movement. Most of the adductor muscle is translucent, indicating the importance of fast contraction. The adductor of the Ostreidae contracts rapidly only in adverse conditions or to clear the mantle of detritus, but the Pectinidae are

TABLE 1. Relative size and type of adductor muscles in species studied.

	Family	Species	Common name	Size of anterior (A) and posterior (P) adductors	Size of opaque (O) and translucent (T) parts of each adductor
Phylum mollusca					
Class Pelecypoda	1 Nuculidae	*Nucula proxima*	Atlantic nut clam		T=O
	2 Nuculanidae	*Yoldia limatula*	File Yoldia		
Protobranchia				A=P	
	3 Mactridae	*Spisula solidissima*	Atlantic surf clam		T>O
	4 Pleurophoridae	*Arctica islandica*	Ocean quahog		T=O
	5 Veneridae	*Venus mercenaria*	Northern quahog		T=O
Shallow-burrowing	6 Astartidae	*Astarte undata*	Waved astarte		T<O
lamellibranchs	7 Carditidae	*Venericardia borealis*	Northern heart shell		T<O
	8 Cardiidae	*Clinocardium ciliatum*	Iceland cockle		T=O
	9 Mytilidae	*Mytilus edulis*	Blue mussel		
		Modiolus modiolus	Northern horse mussel	A<P	
Surface attached		*Modiolus demissus*	Atlantic ribbed mussel		T>O
lamellibranchs	10 Pectinidae	*Placopecten magellanicus*	Giant scallop		
	11 Ostreidae	*Crassostrea virginica*	American oyster	A absent	
	12 Solenidae	*Ensis directus*	Common razor clam	A>P	
Deep-burrowing	13 Myacidae	*Mya arenaria*	Common soft-shell clam	A<P	T absent
and immobile lamellibranchs	14 Hiatellidae	*Hiatella arctica*	Arctic saxicave		

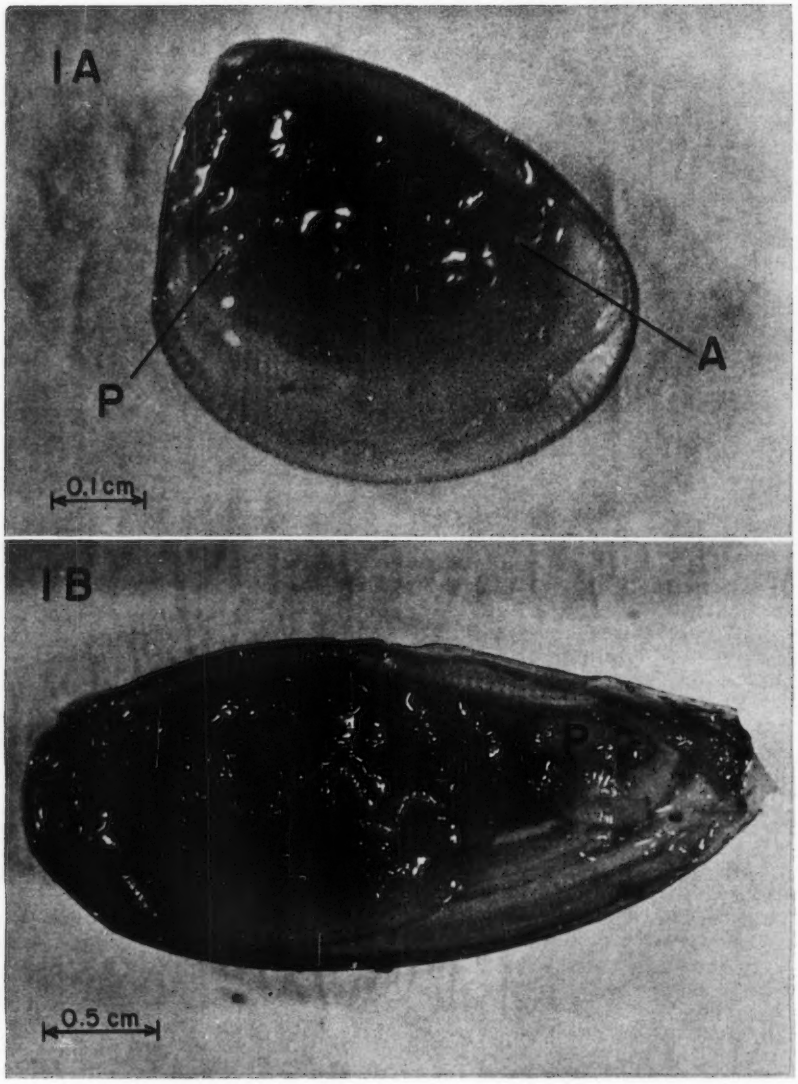

FIG. 1. Protobranchia. A, *Nucula proxima* — The right valve and some internal organs have been removed to show the anterior and posterior adductors, which are of almost the same shape and size; B, *Yoldia limatula* — The left valve has been displaced to show the anterior and posterior adductors, which are symmetrical as in *Nucula proxima*, A = anterior adductor; P = posterior adductor.

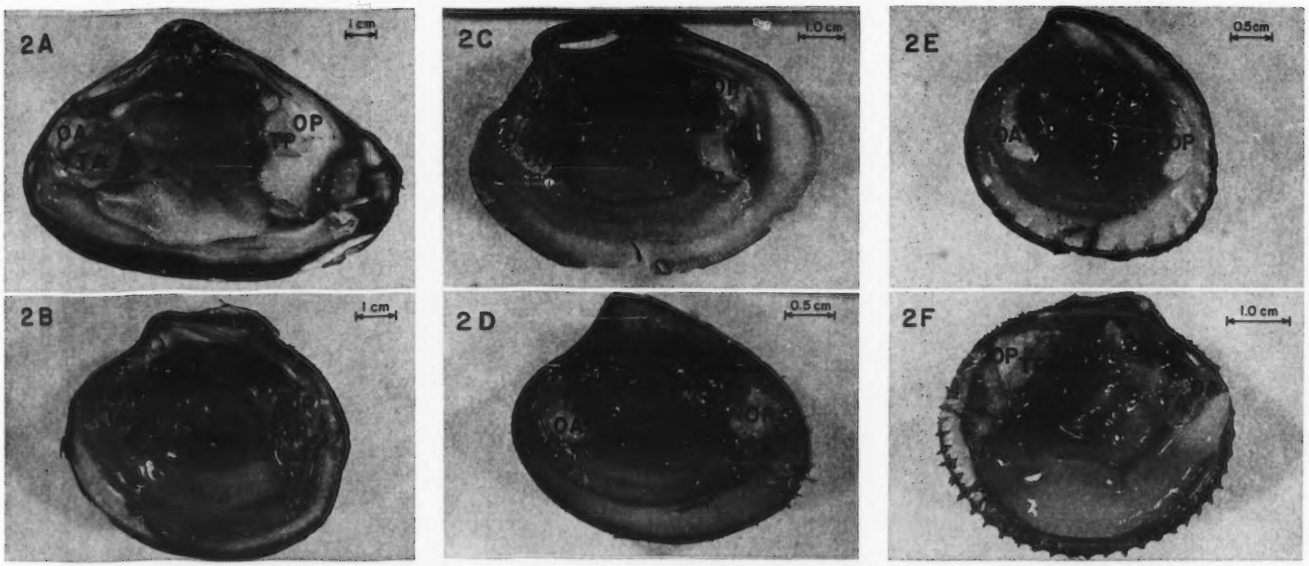

FIG. 2. Shallow-burrowing lamellibranchs. In A–E, the left valve has been removed. A, *Spisula solidissima* — The anterior and posterior adductors, which are of similar shape and size, are divided into a translucent and an opaque portion; the translucent part is the larger; B, *Arctica islandica* — The two moderately symmetrical adductors are divided into almost equal translucent and opaque portions; C, *Venus mercenaria* — The anterior and posterior adductors are fairly symmetrical. The former is divided into translucent and opaque portions almost equal in size; the latter appears to have three portions — opaque, translucent, and of intermediate translucency; D, *Astarte undata* and *E. Venericardia borealis* — In both species, each of the two fairly symmetrical adductors is divided into an opaque portion and a somewhat smaller translucent part; F, *Clinocardium ciliatum* — The right valve has been displaced to show two adductors, fairly equal in shape and size, divided into almost equal translucent and opaque parts. OA = opaque part of anterior adductor; TA = translucent part of anterior adductor; OP = opaque part of posterior adductor; TP = translucent part of anterior adductor; IP = intermediately translucent part of posterior adductor.

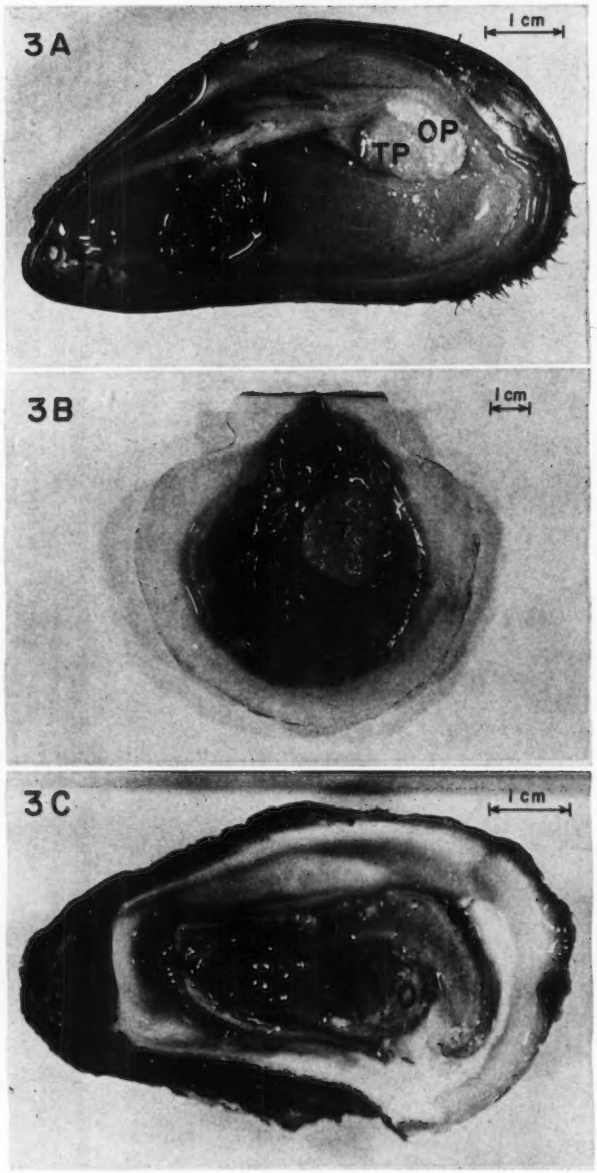

FIG. 3. Surface attached lamellibranchs. The left valve has been removed. A, *Modiolus modiolus* — The anterior adductor is smaller than the posterior one; each is divided into a translucent and smaller opaque portion; B, *Placopecten magellanicus* — There is only one (posterior) adductor, centrally located. The translucent portion is much larger than the opaque part of the muscle; C, *Crassostrea virginica* — This has only one adductor, divided into a translucent portion and a smaller opaque portion.

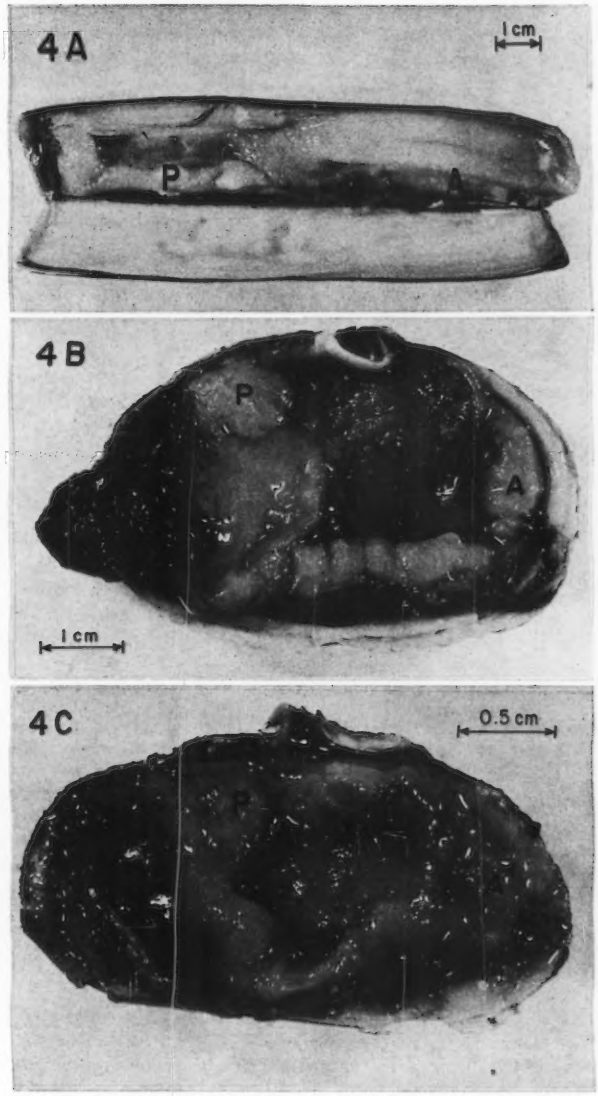

FIG. 4. Deep-burrowing and immobile lamellibranchs. A, *Ensis directus* — The adductors have been cut and the left valve deflected, revealing an elongated anterior adductor, and smaller more rounded posterior adductor. No clear division into translucent and opaque parts is evident, but the posterior part of the anterior adductor is slightly translucent; B, *Mya arenaria* and *C. Hiatella arctica* — The right valve has been removed. The anterior adductor (A) is more elongated than the larger posterior adductor (P). Division into translucent and opaque parts is not apparent.

Morrison and Odense — J. Fish Res. Board Can.

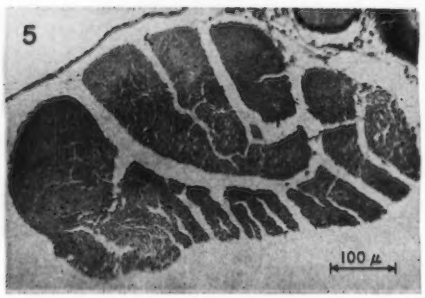

FIG. 5. Transverse section of adductor of *Nucula proxima*. Preparation embedded in paraffin and stained with hematoxylin and eosin. The fibers at the larger end of the adductor (at left) are wider than those at the smaller end.

free from any attachment to the substrate and most of the adductor consists of striated muscle, making swimming movements possible. The smooth muscle may perform the same functions as in the protobranchs and shallow-burrowing lamellibranchs. This would be especially important in intertidal Mytilidae, in which smooth muscle forms a larger part of the adductor than in other surface attached lamellibranchs, and in which maintenance of adduction is an adaptation to avoid the dessicating effect of exposure.

Ensis directus, *M. arenaria*, and *H. arctica*, which are classified in the deep-burrowing and sessile lamellibranchs, have elongated shells that facilitate burrowing. The shape of their anterior adductors reflects the shape of the shells, whereas the posterior adductor is compact. This probably gives more room for the movements used in burrowing. Each adductor does not seem to be clearly divided into two types of muscle. Pelecypods in a deep burrow probably have fewer locomotory reactions than epibenthic molluscs, and may not require two types of adductor muscle.

The increased post-mortem opacity of the translucent parts of the adductors can develop rapidly, so descriptions of adductors can be misleading unless made immediately after death. The change is in refractive index, and probably is caused by denaturation of the proteins during post-mortem changes. As the opacity of the opaque muscle does not change appreciably after death, it then appears more translucent than the part that was translucent in the living organism. Opaque muscle contains more paramyosin than translucent muscle (Szent-Györgyi et al. 1971). The method of isolating paramyosin shows that it is resistant to dehydration and to changes in *p*H and temperature (Philpott et al. 1960; Johnson et al. 1959). The actomyosin system of translucent muscles may be more labile and susceptible to post-mortem changes than the paramyosin that constitutes a large proportion of the opaque muscle.

The results of this study are summarized in Table 1. The gross morphology of the adductors shows that this is a structural character that fits into and supports Morton's classification.

Acknowledgments — C. M. Morrison was in receipt of an NRC scholarship while doing this work. This report formed part of her Ph.D. thesis in biology, Dalhousie University, 1970.

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Postglacial Colonization of the Matamek River, Quebec, by Fishes[1]

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POWER, G., G. F. POPE, AND B. W. COAD. 1973. Postglacial colonization of the Matamek River, Quebec, by fishes. J. Fish. Res. Board Can. 30: 1586–1589.

Since all species of fish in the Matamek River and tributaries are euryhaline and since the number of species present at any location decreases progressively from the mouth to the headwaters, recolonization must have proceeded only from the Gulf of St. Lawrence. The distribution of species in the system, especially the locations of isolated and relict populations, indicates the order in which these species arrived in the system.

POWER, G., G. F. POPE, AND B. W. COAD. 1973. Postglacial colonization of the Matamek River, Quebec, by fishes. J. Fish. Res. Board Can. 30: 1586–1589.

Puisque toutes les espèces de poissons de la rivière Matamec et de ses tributaires sont euryhalines, et que le nombre d'espèces présentes à différents endroits décroît progressivement de l'embouchure à la source, la recolonisation a dû se faire seulement à partir du golfe Saint-Laurent. La distribution des espèces dans le milieu, surtout celle des populations isolées et relictuelles, montre l'ordre dans lequel ces espèces ont occupé le milieu.

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THIS paper records distribution of fishes in the Matamek River, a small (about 684 km^2) north shore, Gulf of St. Lawrence drainage basin. It provides details of the fish fauna in a little known part of Quebec. A hypothesis on postglacial colonization of the Matamek watershed by fishes is developed which includes order of arrival and a marine route of entry. Evidence in support of the hypothesis is provided by distributional data, geomorphological evidence of postglacial changes in sea and land elevations and of barriers to dispersal, and ecology of the species involved. This kind of information is urgently needed for north shore Gulf of St. Lawrence watersheds as many have already been disturbed and manipulated for industrial reasons and others are threatened.

Materials and methods — Between 1967 and 1973 extensive collections of fish were made throughout the drainage basin by more than a dozen students working on various aquatic ecology projects, see for example Saunders (1969), Schiefer (1969), Carscadden (1970), Pope (1970), O'Connor (1971), and Coad (1972). Lakes and suitable parts of the lower river were sampled using gillnets (150 × 6 ft nylon, multifilament, 1.5–4.0 inch mesh, 50 × 6 ft monofilament, 0.5–4.0 inch mesh set from shore to depths of 60 ft and offshore to depths of 300 ft), fykenets, trapnets, and a trammel net. Some smaller streams and branches of the upper river were sampled with rotenone. Other streams were studied using semi-permanent traps of diverse construction, fykenets, dipnets, and dipnets in conjunction with 100–300 candle power lanterns at night. Fly fishing was used to capture both young and adult salmonids and baited long lines took eels. Electro-fishing equipment proved inoperable because of the low conductivity of the water (12–20 μmhos/cm). Littoral areas of the Gulf of St. Lawrence were sampled at various times and intertidal and shallow water fish populations sampled using dipnets, seines, and gillnets.

Present distribution of fishes — Records of fishes collected are summarized in Table 1, locations are identified in Fig. 1. Excluding species making temporary intrusions into the estuary nine species of fish were found above the first falls, a 5-m barrier to penetration upriver by euryhaline species except salmonids and eels. Upstream above successive waterfalls fewer species occur until in some bodies of water in the upper eastern half of the basin only *Salvelinus fontinalis* was found and about one-third of the basin, the upper western portion above an escarpment marking the edge of the Laurentian Plateau, was fishless.

Collections made in a variety of ways over a period of years cannot be precisely quantitative but none the less they provide a valid indication of relative abundance. In Table 1 species designated

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A, abundant or C, common occurred in almost every collection by any type of gear and were numerous. Species designated P, present were either small and inconspicuous or sampling must be directed towards capturing them and even then they were not plentiful. Rare species were those found only occasionally and then only in small numbers.

Collections in the littoral marine area were not intensive but the following species were taken: *Gasterosteus aculeatus*, *Pungitius pungitius*, *Gasterosteus wheatlandi*, *Cyclopterus lumpus*, *Gadus morhua*, *Mallotus villosus*, *Myoxocephalus scorpius*, *Pseudopleuronectes americanus*, *Scomber scombrus*, *Ammodytes americanus*. Of these only *Gasterosteus wheatlandi* is at the northern limit of its range in this part of Quebec.

Postglacial colonization — During the Wisconsin glaciation fish were exterminated from the land mass north of the Gulf of St. Lawrence. Fishes present in the region today have colonized it from refugia along the Atlantic coast and in the Mississippi valley (see McPhail and Lindsey (1970)

TABLE 1. Distribution and abundance of fish species in the Matamek River and littoral environs. Freshwater species are arranged from left to right in presumed order of entry; locations from top to bottom in approximate order of elevation above sea level.

Locale	Species														
	Salvelinus alpinus	*Gasterosteus aculeatus*	*Pungitius pungitius*	*Osmerus mordax*	*Salvelinus fontinalis*	*Salmo salar*	*Anguilla rostrata*	*Catostomus catostomus*	*Apeltes quadracus*	*Alosa pseudoharengus*	*Microgadus tomcod*	*Myoxocephalus scorpius*	*Pseudopleuronectes americanus*
Matamek and Tributaries													
Estuary to First Falls		P	R		C	C	C			P	P	P	P
Below Second Falls		P	C	R	A	A	C	C	P				
Below Third Falls		P	C		A	A	C	C					
Below Fourth Falls		P	P		A	A	R						
Below Fifth Falls	R	P	C		A		R						
Lower Muskrat River		P	P	R	C	P	C	C					
Bill Lake		A	P	A	A		P						
Muskrat Lake		A	C	A									
Matamek Lake	P	A	C	A	A								
C 11			C		C								
C 21		C	P		C								
Cross Lake	P	A	C	C	C								
C 24		C	P	R	C								
C 12			P		C								
C 22		C	P	P	C								
C 23	R	C	P	R	C								
Key Lake		C	P	P	C								
Upper Matamek River		P	P		A	I							
L 11					C								
Claveau Lake					A								
Macrae Lake					A								
Lake X					C								
L 12					A								
L 13					A								
Introductions													
Gallienne Lake						I							
Randin Lake						I							
Upper Muskrat River						I							
LO 2						I							

Symbols

A Abundant

C Common

P Present

R Rare

I Introduced

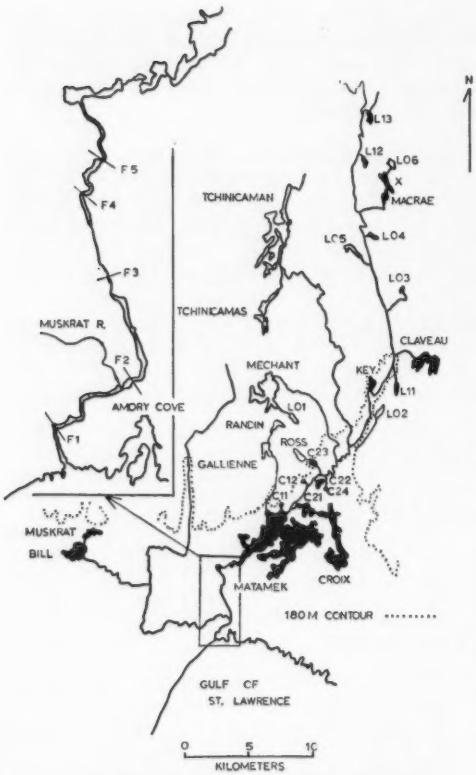

FIG. 1. The Matamek River showing lakes colonized by fish, colored black; position of the waterfalls in the lower river and locations mentioned in the text and Table 1.

for a brief discussion). Retreat of the ice and improvement of the climate was accompanied by changes in sea level and isostatic rebound of the land. At its maximum intrusion inland the Champlain Sea extended up the Matamek River valley as far as Key Lake, 140 m above the present sea level (Greig 1945). Colonization of the river by fish began as ecological conditions became suitable and access was obtained via coastal waters. Early arrivals were Arctic char, *Salvelinus alpinus*, rainbow smelt, *Osmerus mordax*, threespine and ninespine sticklebacks, *Gasterosteus aculeatus* and *Pungitius pungitius*, all of which now maintain populations above the 12-m (5th) waterfall at the 90-m contour interval. Brook trout, *Salvelinus fontinalis*, entered the system before this waterfall became an insurmountable barrier but not in time to penetrate from Bill Lake at 96 m over an 11-m waterfall into Muskrat Lake at 108 m. Muskrat Lake contains both rainbow smelt and sticklebacks which colonized the lake before brook trout arrived. Brook trout dispersed widely through the Matamek basin. They are found in the majority of lakes and all the rivers below the Laurentian Plateau. They inhabit the upper Matamek River over its whole length and ascended precipitous streams flowing off the plateau in the region of Lakes X and Claveau (Fig. 1). Brook trout failed to climb the escarpment marking the edge of the plateau in the western part of the basin. Atlantic salmon, *Salmo salar*, entered the system later, after the formation of the fifth and possibly the fourth waterfall at the 82-m contour. The absence of ouananiche (relict salmon) from Matamek Lake supports this view. The fourth waterfall, 5-m high, marks the upstream limit of anadromous salmon migration today. Longnose suckers, *Catostomus catostomus*, are unable to negotiate any of the present falls on the river and are found only below the third and second falls and in the lower Muskrat above a 5-m fall 0.5 km from its confluence with the Matamek. They must therefore have entered the system before formation of the 7-m second falls at the 15-m contour. A recent arrival was the fourspine stickleback, *Apeltes quadracus*, an isolated population of which occurs below the second falls (Coad and Power 1973). No marine populations of this species were located so that the freshwater population is perhaps a relict from an earlier period of climatic optimum. The American eel, *Anguilla rostrata*, is capable of negotiating obstructions which are barriers to other species. Today its more southerly distribution than the chars, salmon, and sticklebacks suggests it would have been a relatively recent arrival in the Matamek. Today it penetrates the river as far as the fifth waterfall and its tributary the Muskrat River as far as Bill Lake.

The absence of sculpins, *Cottus* sp.; lake trout, *Salvelinus namaycush*; lake whitefish, *Coregonus clupeaformis*; round whitefish, *Prosopium cylindraceum*; northern pike, *Esox lucius*; lake chub, *Couesius plumbeus*; longnose dace, *Rhinichthys cataractae*; all of which could reasonably be expected to thrive in the watershed, together with the pattern of distribution of species which occur, is ample testimony to the difficulty of colonizing a small northern river basin such as the Matamek. While small rivers have been colonized via the sea, access to large rivers on the north shore of the Gulf of St. Lawrence has also been possible from the interior by headwater capture and other changes in drainage (Power 1965). These rivers contain all or most of the species listed above as absent in the Matamek.

Acknowledgments — We would like to thank the staff and students of the Matamek Research Station, Que., for their various contributions to this paper. This research was financed by the Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543 and the Atlantic Foundation.

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Use of a Silver-Sulfide Electrode for Standardizing Aqueous Sulfide Solution in Determining Sulfide in Water

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BARICA, J. 1973. Use of a silver-sulfide electrode for standardizing aqueous sulfide solution in determining sulfide in water. J. Fish. Res. Board Can. 30: 1589–1591.

A simplified method is described for standardizing aqueous sulfide solution in determining sulfide in water. A silver-sulfide electrode is used to detect the endpoint in titration of standard solution of sodium sulfide by silver nitrate. The method makes it possible to perform potentiometric titration within 60 sec by adding the titrant until the potential reading is between –150 and 0 mv, without plotting the data on a graph. The concentrations of sulfide solutions determined by this method were within ± 0.24 mg S (SD), or 3%, of the values obtained by the conventional iodometric standardization.

BARICA, J. 1973. Use of a silver-sulfide electrode for standardizing aqueous sulfide solution in determining sulfide in water. J. Fish. Res. Board Can. 30: 1589–1591.

L'auteur décrit une méthode simplifiée de standardisation d'une solution aqueuse de sulfure pour le dosage du sulfure dans l'eau. Une électrode à sulfure d'argent est utilisée pour déterminer le point de virage dans le titrage de la solution standard de sulfure de sodium par le nitrate d'argent. La méthode permet un titrage potentiométrique en moins de 60 sec, en ajoutant le produit de titrage jusqu'à ce que le potentiel se lise entre –150 et 0 millivolts, sans porter les données sur un graphique. Les concentrations de solutions de sulfure déterminées par cette méthode se situent dans les limites de ± 0.24 mg S (SD) ou 3% des valeurs obtenues par standardisation iodométrique conventionnelle.

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SPECTROPHOTOMETRIC determination of hydrogen sulfide or total sulfide in natural waters using *p*-phenylendiamine and ferric chloride is a well established method. It poses, however, a certain drawback concerning standardization. As the standard sulfide solution has to be prepared from $Na_2S \cdot 9H_2O$ containing a large amount of water of hydration, precise standards cannot be prepared by weighing the salt. Usually, an approximate solution is standardized by back titration of added iodine or iodate solution with thiosulfate, using starch as indicator (Strickland and Parsons 1968; APHA 1971).

This report describes use of a silver-sulfide electrode in standardizing the sulfide solution. The method is more rapid than and as precise as the conventional iodometric technique.

Outline of the Method — The silver-sulfide electrode currently available from various manufacturers is an electrochemical sensor that can be used to measure the activity or concentration of free sulfide and silver ions in solutions. Since they form an insoluble silver sulfide, in effect only one of them can be present in a solution. The electrode has a solid-state sensing element and can be used like a conventional *p*H electrode in combination with a suitable reference electrode. The sensing element is a solid silver sulfide membrane which allows silver ions to pass between the sample solution and the internal reference solution, which is kept at a fixed silver concentration. The distribution of silver ions between the two solutions develops a potential which depends on the silver ion activity in the sample solution and is given by Nernst's equation (Pungor 1967; Orion 1970).

Although the electrode can detect very low levels of sulfide ion, it is, like any specific ion sensor, a logarithmic device subject to various interferences as well as temperature and electrode drift, and requires a constant ionic strength background if low concentrations of sulfide ions are to be detected. Moreover, it senses only dissociated sulfide anion and not HS^- or undissociated H_2S concurrently present in solution. Its use for direct determination of total sulfide in water is therefore limited.

However, the unique capacity of the electrode to detect both Ag and S ions makes it a highly sensitive endpoint detector for titration of a sulfide sample of higher concentrations when $AgNO_3$ is used (Hseu and Rechnitz 1968; Mascini and Liberti 1970) as the titrant and hence an aid in standardizing of the spectrophotometric method. Since the titration is carried out in distilled water, all interferences are eliminated and the electrode indicates the first appearance of free Ag ions after precipitating all sulfide in the solution.

Figure 1 shows a typical curve for titration of 5 and 10 mg S from standard solution by 0.0624 N $AgNO_3$ (normality of silver nitrate solution adjusted so that 1 ml of the titrant corresponded to 1 mg S). The large break of about 1100 mv makes it unnecessary to find the endpoint by plotting or drawing a derivation curve. Since the endpoint lies near −150 mv, it is sufficient to titrate until the potential reading is about that value, which usually takes less than 1 min. Even by exceeding this point for as much as 300 mv (to +150 mv), the error by overtitration is only +1.2% for 10 mg S and +2.2% for 5 mg S. The large potential change makes it possible to take a reading directly with any *p*H or millivolt meter without using an expanded scale.

Since the stock sulfide solution is unstable with time (Fig. 2), standardization must be carried out before each batch of samples.

The values obtained by this method were compared with those from the conventional iodometric standardization using thiosulfate and iodine solutions and starch as the endpoint indicator (Strickland and Parsons 1968). The mean concentrations of the standard sulfide solution (10 for each test) were within ±0.13 mg (SD; 1.6%) at the 5 mg S level and ±0.24 (3%) at the 10 mg S level of the mean values obtained by iodometric standardization.

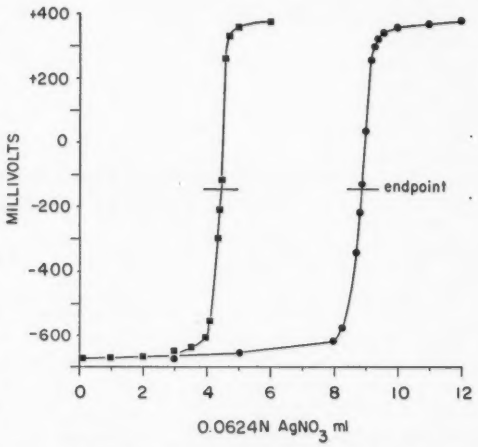

FIG. 1. Typical titration curves for 50 (*left*) and 100 ml (*right*) of standard sulfide solution (approximately 5 and 10 mg S).

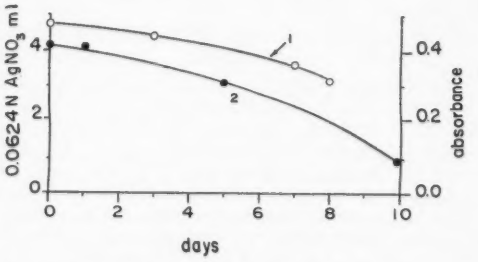

FIG. 2. Stability of standard sulfide solution. 1, 5 mg S, titrated by 0.0624 N $AgNO_3$; 2, 300 μg/liter S, determined spectrophotometrically.

Equipment — 1, Silver-sulfide ion electrode (Orion Model 94–16 or comparable specific ion electrode sensitive to silver or sulfide ions); 2, Reference electrode with filling solution other than KCl (Orion Model 90–02 Double Junction Reference Electrode with 1 M KNO_3 in outer sleeve or comparable electrode); 3, *p*H meter with a millivolt scale (Orion Model 407 Specific Ion Meter or a comparable millivolt meter); 4, Magnetic stirrer; 10-ml microburette.

Reagents — 1, Silver nitrate solution, 0.0624 N. Dry pulverized reagent grade $AgNO_3$ at 150 C for 24–48 hr. Dissolve 10.595 g of the dried reagent in 1000 ml of deoxygenated distilled water. Store in a dark bottle. One milliliter of this solution corresponds to 1 mg S; 2, Stock sulfide solution. Dissolve approximately 7.5 g $Na_2S{\cdot}9\ H_2O$ in 1000 ml of deoxygenated water. Weigh out only the driest and cleanest looking crystals. 1 ml = approximately 1 mg S; 3, Standard sulfide solution. Pipet 10 ml of the stock sulfide solution into a 100-ml volumetric flask and make up to the mark by deoxygenated water. 1 ml = approximately 0.1 mg S; 4, Deoxygenated distilled water. Bubble distilled water vigorously with nitrogen gas for 30 min. Store under nitrogen atmosphere. If nitrogen gas is not available, boil and cool distilled water before use.

Procedure — 1, Take exactly 1.0 ml of standard sulfide solution and make up to 100 ml with deoatxygened distilled water. Take an aliquot and add colorimetric reagents, following procedures described elsewhere (Strickland and Parsons 1968; Cline 1969; APHA 1971). After the required time, read the absorbance. Concentration of this solution is approximately 1000 μg/liter S. Run in duplicate; 2, Without delay pipet 50.0 ml of standard sulfide solution into a beaker. Place both electrodes into the solution and connect mv meter. Add 0.0624 N $AgNO_3$ solution from a microburette in increments of 0.5–1.0 ml in the beginning of the titration. Stir the solution continuously. When the potential starts to increase to about –600 mv, add the titrant in 0.05-ml increments until the reading is between –150 and 0 mv. Note the volume of titrant which has been added to the solution (about 5 ml). Calculate the correction factor, C, of sulfide in the stock solution:

$$C = \frac{V}{5.00}$$

where V is the volume of titrant added.
After reading the absorbances of colorimetric standards, the slope factor, F, can be calculated as

$$F = \frac{1000 \cdot C}{A_c}$$

where A_c is the mean net absorbance corrected for the reagent blank. Concentration of sulfide, S_d, in an unknown sample is then

$$S_d = A_s \cdot F\ \mu g/liter\ S$$

where A_s is net absorbance of the unknown sample.

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Vocalization in the Ringed Seal (*Phoca hispida*)

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STIRLING, I. 1973. Vocalization in the ringed seal (*Phoca hispida*). J. Fish. Res. Board Can. 30: 1592–1594.

The ringed seal (*Phoca hispida*), previously thought to be silent, was found to emit vocalizations under water that were not readily audible to man through the ice. The four vocalizations identified (high- and low-pitched barks, yelps, and chirps) probably facilitate communication and social organization.

STIRLING, I. 1973. Vocalization in the ringed seal (*Phoca hispida*). J. Fish. Res. Board Can. 30: 1592–1594.

On a constaté que le phoque annelé (*Phoca hispida*), considéré dans le passé comme silencieux, pouvait émettre des sons articulés sous l'eau qui n'étaient pas facilement entendus par l'homme sur la surface de la banquise. Les quatres vocalisations identifiés (jappements aigus et graves, glapissements et pépiements) facilitent probablement la communication et l'organisation sociale.

Received March 16, 1973

THIS study was made to determine whether or not the ringed seal (*Phoca hispida*) vocalizes beneath the ice. The ecological counterpart of the ringed seal in the Antarctic, the Weddell seal (*Leptonychotes weddelli*) (Stirling 1969), makes numerous loud underwater vocalizations that are readily audible to a human on the ice. The ringed seal has been reputed to be silent. Eskimo hunters were aware of the "trilling" of the bearded seal (*Erignathus barbatus*) but knew of no calls originating from ringed seals, nor had biologists studying the ringed seal in the field ever heard underwater vocalizations (A. W. Mansfield, I. A. McLaren, and T. G. Smith personal communication) although a faint underwater "click" which was inaudible to the unaided human ear had been recorded from a captive ringed seal (Schevill et al. 1963). Specific recognition of underwater vocalizations of arctic pinnipeds will aid in projected studies of sub-ice distribution and social behavior.

Materials and methods — A winter trip was made to record vocalizations made outside the breeding season (April–May). On January 20 and 21, 1972, in an area with several ringed seal breathing holes on the west coast of Victoria Island, a hydrophone was lowered down a breathing hole and monitored for a total of 16 hr within a 24-hr period. Two hundred and forty minutes of recordings were made. I was confident that any vocalizations heard would originate from ringed seals because bearded seals were not common in the area at any time of year and less so during winter. The identity of the vocalizations has since been confirmed from captive ringed seals (K. Ronald personal communication).

From March 28, to May 2, 1972, I recorded seal vocalizations in many locations in Amundsen Gulf and the Beaufort Sea. Recordings that included the distinctive call of the bearded seal (Ray et al. 1969) were discarded. In addition, when bearded seals were not heard, the presence of ringed seals was often confirmed by looking down into the water through the breathing hole. One hundred and twenty-four minutes of recording from this period were analyzed.

From May 31, to June 12, 1972, T. G. Smith recorded with the same equipment in the vicinity of some active birth lairs and adjacent open leads in Prince Albert Sound. He allowed me to analyze his tapes.

During August I recorded in open water west of Sachs Harbour, Banks Island, in areas where ringed seals had been seen. Two hundred minutes of recording were analyzed.

Underwater recording was done with a Uher 4000L Report tape recorder using Ampex and BASF tapes and a LC-50 hydrophone with an LG-1324 amplifier (Atlantic Research Corporation).

Results and discussion — Four vocalizations made by ringed seals were heard at all times of the day and night in all seasons and in all areas. Most common were low-pitched barks, given singly or in a series, and a high pitched (sometimes modulated) yelp or yowl (Fig. 1a). Also heard, but much less frequently, were low and high pitched growls (Fig. 1b) and a short descending chirp (Fig. 1c). No calls that could be associated with echo location were identified.

The functions of these calls may be suggested. The bark is probably a direct threat as similar

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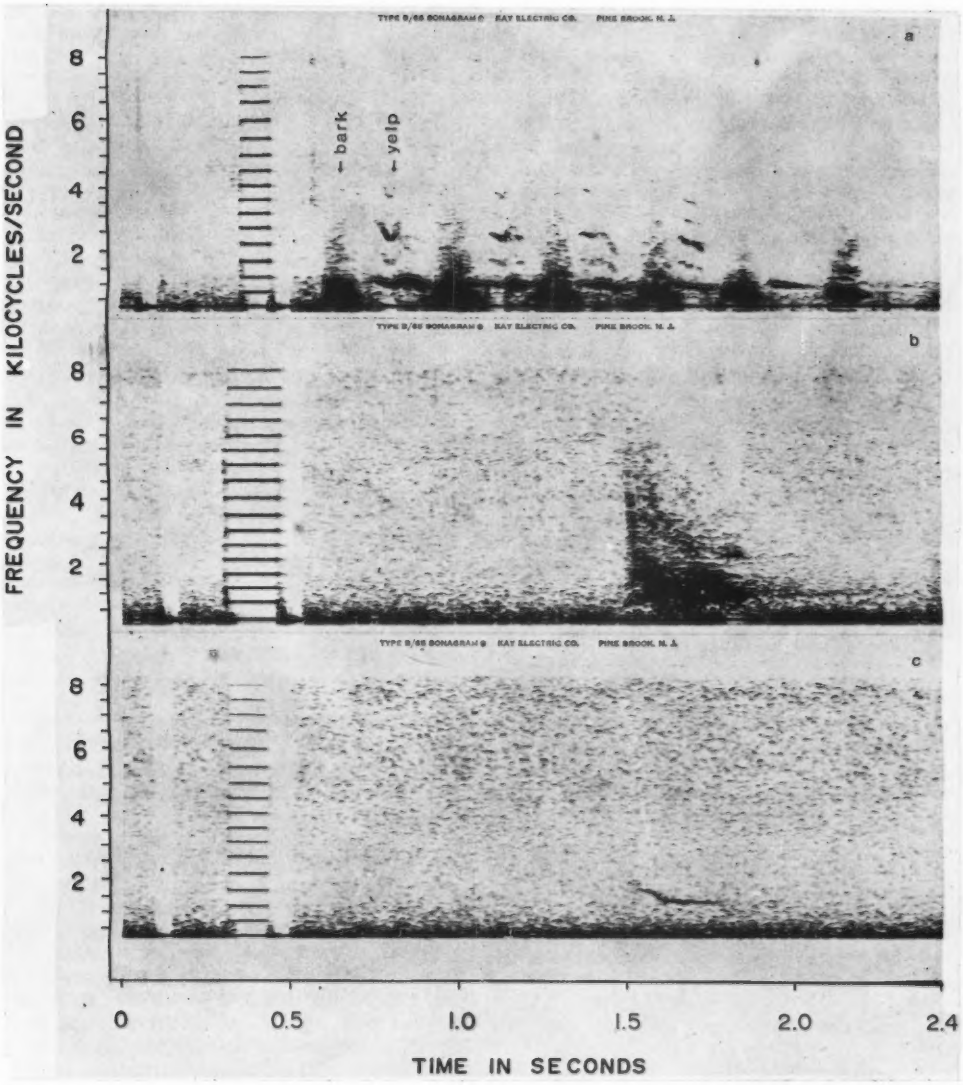

FIG. 1. Sonograms of ringed seal vocalizations: (a) barks and yelps, (b) high-pitched growl, (c) descending chirp.

Stirling — J. Fish. Res. Board Can.

threats are given by the males of several other species of pinnipeds. For example, the physical structure and frequency in kilocycles of the threatening underwater bark of the California sea lion (*Zalophus californianus*) described by Schusterman and Balliet (1969) are apparently similar to those of the bark of the ringed seal.

The high-pitched yelp, which is sometimes modulated, is probably a submissive call. Similar vocalizations are used by other pinnipeds and canids as submissive displays (eg. the South Australian fur seal (*Arctocephalus forsteri*), as described and illustrated by Stirling and Warneke 1971). The bark and yelp were often heard in response to each other, apparently during an agonistic encounter.

The growl is probably also a threat as it was also often heard in apparent association with the yelps. The low-pitched growl was more common than the higher-pitched growl illustrated in Fig. 1b. (None of the sound spectrographs of the low-pitched growl were suitable for reproduction).

The function of the descending chirp (Fig. 1c) is uncertain although it occurs over a much more restricted frequency range. It has a structural similarity to the descending trill of the Weddell seal, which Kooyman (1968) illustrated and assigned to a threatening role.

In the spring, all recorded calls known to have originated from ringed seals were similar to those recorded during the winter. Thus, no vocalizations which played a role solely in reproductive behavior were identified.

The only apparent difference in vocalizations between the winter and spring samples was that the frequencies of the barks and yelps were reversed. Of 633 vocalizations recorded in the winter, deep barks and yelps comprised 61.5 and 30.0%, respectively compared to 24.4 and 61.0%, respectively of 364 vocalizations recorded in the spring. The difference may be related to a probable higher level of aggressiveness of adults during the breeding season. Aggressive behavior between wild ringed seals in April becomes intense enough to result in younger animals being excluded from the water long enough to be frozen out. Considering the ringed seal's reputation for alertness when basking on the sea ice, and how easily it may be frightened into the water, it is apparent that considerable social pressure must exist in the water to cause a subadult to remain on the sea ice, where it is vulnerable to predation, for long enough to become cut off from safety and food supply by freezing. All three such subadults that I examined had many bite wounds in the axilla and chest areas characteristic of phocid intraspecific fighting (eg. Smith 1966). In the highly territorial and aggressive South Australian fur seal, subadults giving the modulated submissive call may be permitted to cross a territory unscathed if they do so quickly (Stirling 1971). Similarily, subdominant ringed seals likely increase their submissive vocalizing in spring compared to winter simply to be permitted to travel through a volume of water that might be defended.

From winter to spring there was a 11% increase in the number of vocalizations/minute, from 2.64 to 2.94. This may also be related to an increased level of agonistic behavior during the breeding season.

During August, a total of only 25 vocalizations were recorded (0.125 vocalizations/min) in 200 min of monitoring the open water. Since both ringed and bearded seals were present, I could not be certain which species gave the calls heard. However, it is still obvious that the amount of vocalizing was less. This may be the product of two factors: during conditions of ice cover seals may concentrate at breathing holes and leads, necessitating more vocalizing to maintain social order; and during the open water season, they presumably are more widely dispersed and vocalize less as well.

Thus, the ringed seal was found to be a vocally active species, even though the calls were not readily audible to a man on the surface of the sea ice.

Acknowledgments — I am particularly grateful to the following: T. G. Smith of the Arctic Biological Station, Fisheries Research Board of Canada, for use of laboratory facilities at Holman, N.W.T., access to his tape recordings of ringed seals, and assistance in the field; J. Memorana of Holman, N.W.T. for assistance in the field; A. W. Mansfield, Arctic Biological Station, Ste. Anne de Bellevue, I. A. McLaren, Dalhousie University, Halifax, N.S., K. Ronald, University of Guelph, Guelph, Ont., and T. G. Smith for permission to use unpublished observations; and the Dep. of Linguistics, Faculty of Science, University of Alberta, Edmonton, Alta. for permission to make sound spectrographs on their machine.

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Apparatus for Recording Avoidance Movements of Fish

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SCHERER, E., AND S. NOWAK. 1973. Apparatus for recording avoidance movements of fish. J. Fish. Res. Board Can. 30: 1594–1596.

An apparatus is described for continuous measurements of movements of fish exposed to chemical substances in a tank. A strip chart recording provides information on position and rate of movement of the test specimen during the experiment.

SCHERER, E., AND S. NOWAK. 1973. Apparatus for recording avoidance movements of fish. J. Fish. Res. Board Can. 30: 1594–1596.

Les auteurs décrivent un appareil servant à la mesure continue des mouvements de poissons exposés à des substances chimiques dans un bassin. Un enrégistrement sur bande mobile indique la position et le taux de déplacement des spécimens pendant l'expérience.

Received January 19, 1973

THERE is a need for an easily reproducible, reliable method for investigating problems such as: will a fish be attracted or repelled by a certain substance in the water; if so, at what concentration; is the threshold for avoidance above or below a value that would be detrimental or lethal after some time?

We wish to describe and discuss a system that we have found to be practical for standardized tests. Its main elements are: a test chamber similar in principle to those described by Jones (1947) and Sprague (1964), and a novel device for recording the movements of the test specimen under test.

Test chamber (*Fig. 1A*) — Water is pumped into a rectangular tank of clear acrylic plastic from both ends at the same rate, regulated by PVC valves. Our model measures 120 × 18 × 15 cm. Baffles of perforated PVC reduce water turbulence. The water drains from bottom plate and side walls at the center of the tank. Flow velocities of up to 1 cm/sec yield distinct separation between the two bodies of water at the center. A stock solution of the compound to be tested is kept at the same temperature as the water, aerated if appropriate, and injected into either side of the tank by a dosing pump. A row of rheostat-controlled incandescent bulbs and a diffusing screen provide equal illumination of variable intensity. The tank is enclosed and viewed through a one-way mirror to reduce visual stimulation of the fish.

Recording (*Fig. 1B, 2, and 3*) — An open sight viewer, connected to the shaft of a power-supplied potentiometer, is mounted so that an observer can track a specimen in the test chamber. Varying the position of this viewing device changes the output voltage from the to a strip chart recorder, giving an instantaneous, permanent record of fish position and movement, and (2) to a voltage sensitive switch. The latter activates a timer whenever the fish enters one half of the tank, thus giving the cumulative time of exposure to the conditions in that section. A second timer is used to record overall elapsed time of the experiment. The viewing device must be mounted far enough away from the tank so that perspective distortion is minimized; otherwise, correction

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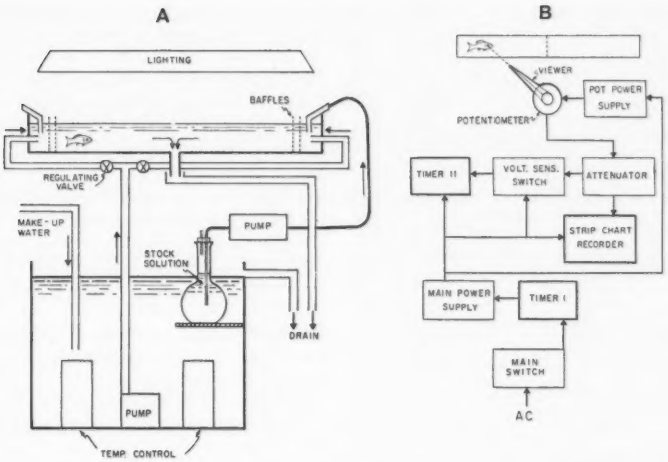

FIG. 1. A, Test chamber with accessories, schematic; B, Block diagram of recording assembly.

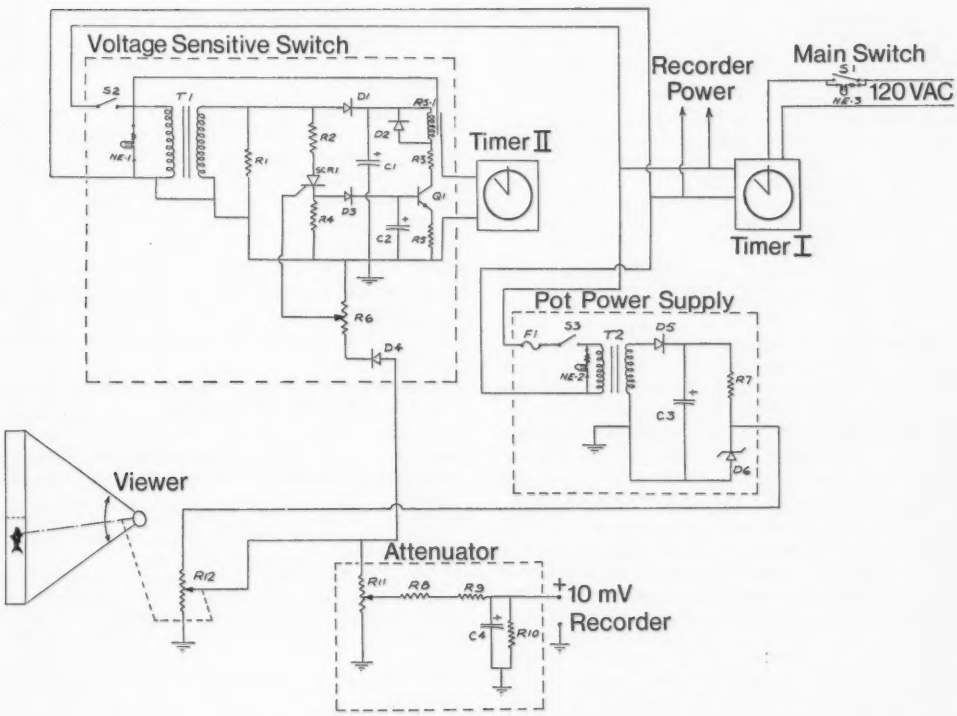

FIG. 2. Circuit diagram of recording assembly.

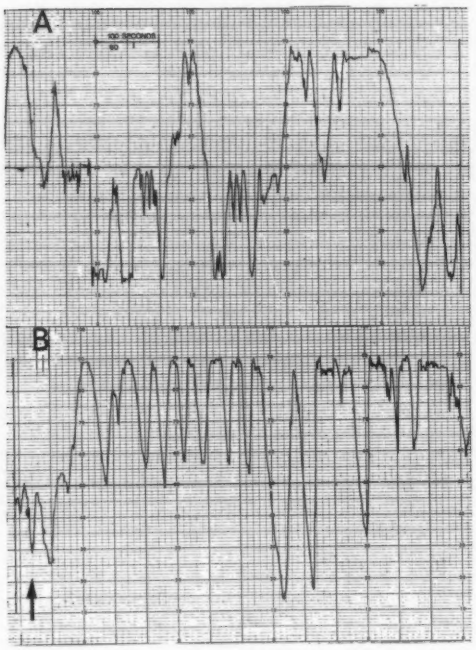

FIG. 3. Recording sample: movements of a goldfish during an experiment. 10 on the chart represents one end of the test area, 50 the middle, and 90 the other end.
A, Clean water in both halves;
B, After application of $HgCl_2$ into side 10–50. Concentration in this section: 2×10^{-4} M.

factors should be applied. An isosceles triangle of tank ends and viewer is recommended.

Discussion — Rectangular chambers cause less optical distortion than tubular ones, which Jones (1947) and Sprague (1964) used, and can be built in any size suited for different species and life stages. We see no reason why aquatic organisms other than fish (e.g. crustaceans, insect larvae) could not be tested with this technique as well. One essential and limiting factor is the mobility of the test animal: the more active it is, the clearer the result. This renders sluggish species or individuals unsuitable. Major external factors determining locomotor activity, namely temperature, oxygen concentration, and light intensity, were made controllable in our system. Temperature of stock solution and water can be kept equilibrated as indicated in Fig. 1A if the rate of make-up water approximates or equals the rate of draining; otherwise, water from the reservoir can be circulated through a waterbath containing the vessel of stock solution.

Recording has been a major problem with previous authors analyzing avoidance reactions. From Shelford and Allee (1913) to Jones (1947) graphs were drawn from notes written intermittently during the experiments. Continuous records were first obtained by Sprague (1964). His method was to manually mark a kymograph while watching the fish. The advantage of our device is its inherently greater accuracy. Very small changes of position can be documented, as shown in Fig. 3: movements as small as 0.5% of the total distance from one end of the test area to the other, equalling 5 mm, are recorded. Given exact positions, the the slopes of trace lines can be used to accurately calculate velocities of the fish. Optimal resolution is achieved by varying the speed of the recorder. During the second half of recording B in Fig. 3, a sequence of three forays into the contaminated area occurs. The average swimming speed of the fish while moving out of this section is about twice that of moving into it (5.2 and 2.5 cm/sec, respectively).

The cost of materials with all parts depicted in Fig. 1A and 1B amounts to $1350. The most expensive single component is a precision dosing pump priced at $670; a cheaper substitute may be sufficient in many cases. "Temp. Control" in Fig. 1A only indicates two stainless steel coils. The recording part is $300; this includes a strip chart recorder for $160 (bought in kit form). Man-hours for construction and assembling are estimated at 50. A package with details for construction and a list of parts and suppliers is available on request.

Acknowledgments — We thank Dr D. P. Scott for his help in revising the manuscript.

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Redescription of *Gaetanus intermedius* Campbell (Calanoida: Copepoda) from the Type Locality

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PARK, T. 1973. Redescription of *Gaetanus intermedius* Campbell (Calanoida: Copepoda) from the type locality. J. Fish. Res. Board Can. 30: 1597–1600.

Gaetanus intermedius Campbell 1930, is redescribed from specimens taken in the type locality, the Vancouver Island region in the northeastern Pacific. *Gaetanus simplex* Brodsky 1950, is found to be a junior synonym of *G. intermedius*.

PARK, T. 1973. Redescription of *Gaetanus intermedius* Campbell (Calanoida: Copepoda) from the type locality. J. Fish. Res. Board Can. 30: 1597–1600.

L'auteur redécrit *Gaetanus intermedius* Campbell 1930, à partir de spécimens capturés dans la localité type, la région de l'île Vancouver, dans le nord-est du Pacifique. Il constate que *Gaetanus simplex* Brodsky 1950, est un synonyme junior de *G. intermedius*.

Received April 3, 1973

CAMPBELL'S (1930) original description of *Gaetanus intermedius*, from one specimen, was so incomplete that it has not been possible to recognize the species with certainty. Brodsky (1950) distinguished the species from his new species, *Gaetanus simplex*, by certain unreliable characters. Vervoort (1957) suggested that *G. intermedius* is synonymous with *Gaetanus minor* Farran.

During the examination of plankton samples collected in the Vancouver Island region, the type locality of *G. intermedius*, a large number of specimens of a species of *Gaetanus* were found. Although larger than Campbell's specimen (2.1 mm), they seemed to be referable to *G. intermedius*, for this is the only species of the genus known to occur in the area and no other species of *Gaetanus* was found during this study in the whole area including the Washington Sound, Strait of Georgia, and Queen Charlotte Island area. Campbell's specimen appeared to be a fifth copepodid.

The following redescription of the species was made from specimens obtained from Washington Sound in the summer of 1965 and confirmed by examining specimens from the Strait of Georgia and Queen Charlotte Island area.

Female — Body length based on 20 randomly selected specimens, 3.20–3.51 mm. Proportional lengths of prosome and urosome about 81:19. Forehead with a small cephalic spine, pointing obliquely forward when viewed laterally (Fig. 1C). Frontal eminence bearing suprafrontal sensilla small. Rostrum single, with a minutely bifurcated tip (Fig. 1D). First metasomal segment and cephalosome fused, as well as fourth and fifth metasomal segments. Posterolateral corner of metasome with a spiniform process, extending a little beyond middle of genital segment. Dorsally, genital segment symmetrical, with moderately swollen lateral side. Laterally, genital prominence pronounced, with small spermathecae.

Antennule reaching to about distal end of genital segment. Second exopodal segment of antenna (Fig. 1F) with a large internal process carrying a seta. Basis of mandible (Fig. 1G) with two subequal setae. Maxillule (Fig. 1H) with 14 setae on first, four each on second and third inner lobes, five on basis, 14 on endopod, 11 on exopod, and nine on outer lobe. Maxilla (Fig. 1I) typical of the genus. Coxa of maxilliped (Fig. 2A) without a lamelliform outgrowth. First two exopodal segments of first leg fused (Fig. 2C), without a trace of joint. Endopod of second leg 2-segmented (Fig. 2D). Third and fourth legs with usual 3-segmented exopods and endopods (Fig. 2E,F). Coxa of fourth leg with a row of spiniform structures on internal margin (Fig. 2G).

Male — Body length based on 20 randomly selected specimens, 2.65–3.10 mm. Proportional lengths of prosome and urosome about 75:25. Cephalic spine small, pointing obliquely forward (Fig. 2J), or absent. Posterolateral corner of metasome with a spiniform process, short of reaching distal end of genital segment. Two rami of antenna about equal in length. Mouth part appendages much reduced, as in the genus. First two exopodal segments of first leg fused, with a vestigial external spine. Coxa of fourth leg without spiniform structures on internal margin. Fifth pair of legs asymmetrical (Fig. 2K), each with 1-segmented endopod. In right leg, endopod short of reaching middle of first

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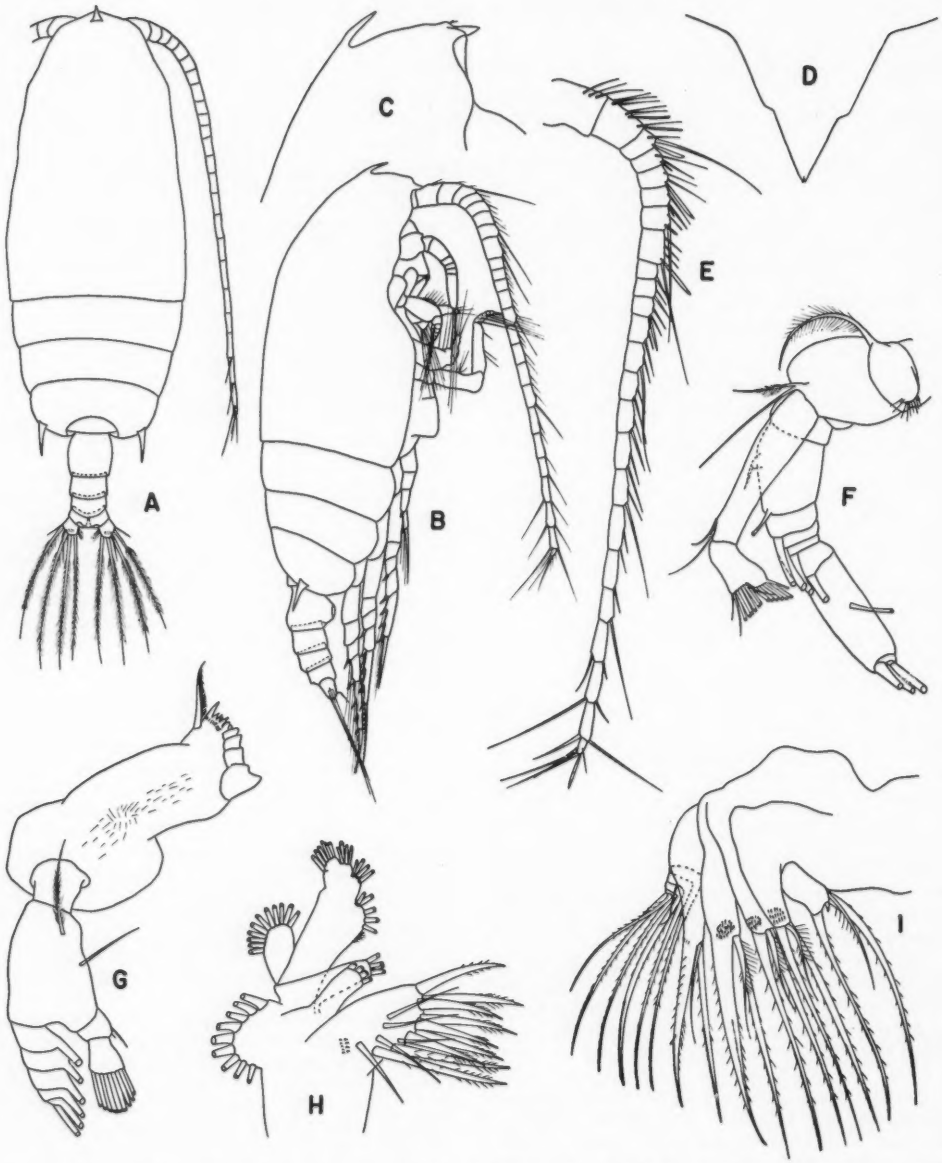

FIG. 1. *Gaetanus intermedius*. Female: A, habitus, dorsal; B, *idem*, lateral; C, forehead, lateral; D, rostrum; E, antennule; F, antenna; G, mandible; H, maxillule; I, maxilla.

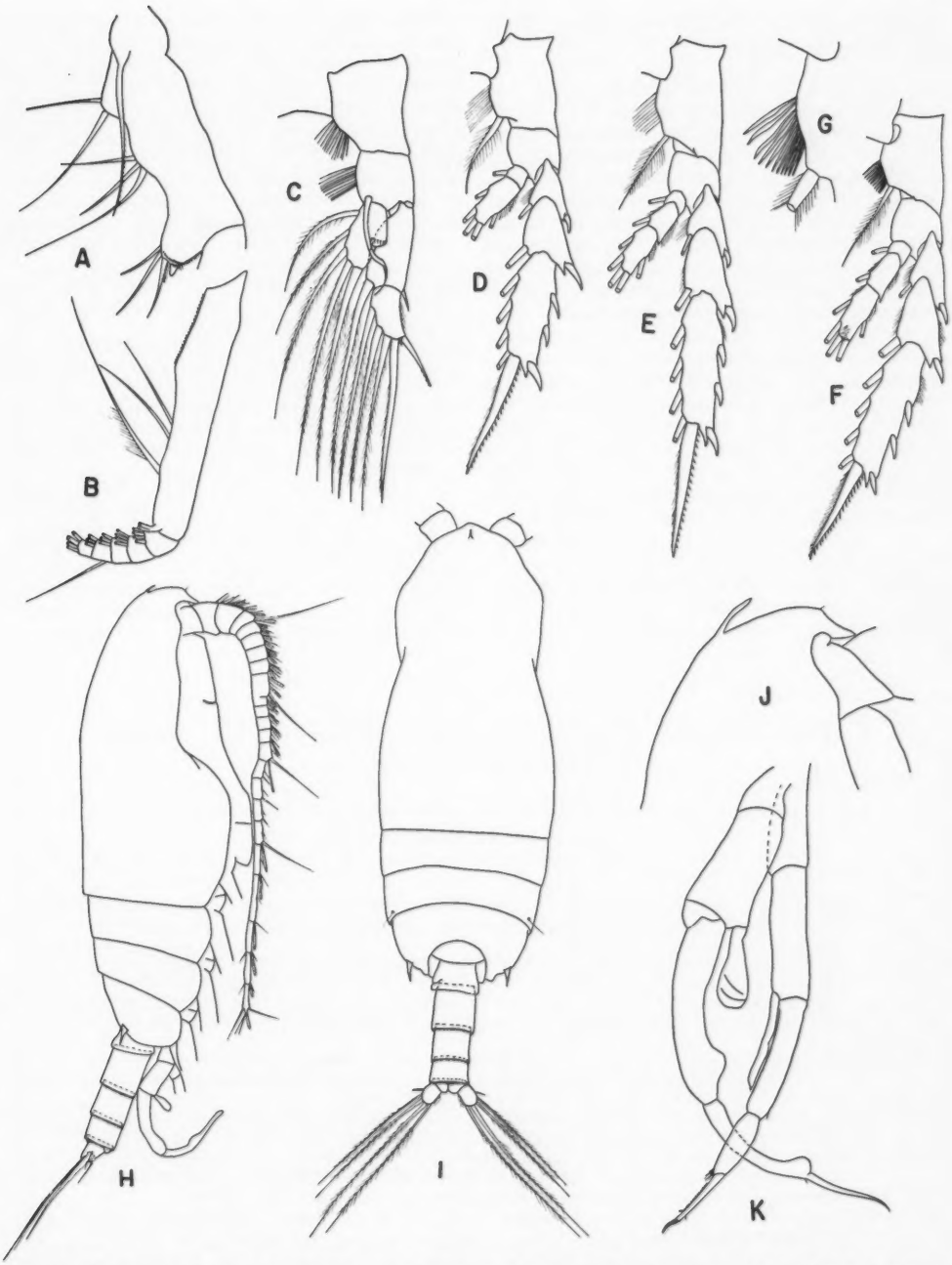

FIG. 2. *Gaetanus intermedius*. Female: A, coxa of maxilliped; B, distal part of maxilliped; C, first leg, anterior; D, second leg, anterior; E, third leg, anterior; F, fourth leg, anterior; G, inner side of coxa of fourth leg. Male: H, habitus, lateral; I, *idem*, dorsal; J, forehead, lateral; K, fifth pair of legs, anterior.

exopodal egment. In left leg, endopod about 4/5 length of first exopodal segment.

Remarks — With a small cephalic spine and without a lamelliform outgrowth on the coxa of the maxilliped, *Gaetanus intermedius* is closely related to *Gaetanus armiger* Giesbrecht, but can be readily distinguished from it by the complete fusion of the first two exopodal segments of the first leg. In *G. armiger*, these two segments are clearly separated (Giesbrecht 1892, pl. 14, fig. 28). In habitus, *G. intermedius* resembles *Gaetanus minor* Farran but differs from it in lacking a lamelliform outgrowth on the coxa of the maxilliped.

Gaetanus simplex Brodsky 1950, described from the Seas of Japan and Okhotsk and Bering Sea, and subsequently synonymized with *G. armiger* by Tanaka and Omori (1970), was examined on the basis of specimens from the type locality and found to be synonymous with *G. intermedius*. The record of *G. armiger* by Davis (1949) from the northeastern Pacific should, according to his descriptions of the first leg, be referred to *G. intermedius*.

As it has not been possible at locate Campbell's original specimen, one of the female specimens from Washington Sound has been deposited as a neotype, along with five additional specimens, in the U.S. National Museum (143775 and 143776).

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Does Eyestalk Ablation Accelerate Molting in the Lobster *Homarus americanus*?

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SOCHASKY, J. B., D. E. AIKEN, AND D. W. MCLEESE. 1973. Does eyestalk ablation accelerate molting in the lobster *Homarus americanus*? J. Fish. Res. Board Can. 30: 1600–1603.

Contrary to published results obtained with larger *Homarus americanus*, bilateral eyestalk ablation induces precocious molting in lobsters about 60 mm carapace length. The observed "acceleration" appears to result from the prevention or termination of a D_0 development plateau. However, limb regeneration in eyestalkless lobsters appears to be inhibited.

SOCHASKY, J. B., D. E. AIKEN, AND D. W. MCLEESE. 1973. Does eyestalk ablation accelerate molting in the lobster *Homarus americanus*? J. Fish. Res. Board Can. 30: 1600–1603.

Contrairement aux résultats déjà publiés obtenus avec de gros *Homarus americanus*, l'ablation des deux pédoncules oculaires provoque la mue précoce chez des homards d'environ 60 mm de longueur de carapace. Cette "accélération" semble résulter de la prévention ou de la terminaison d'un plateau du développement au stade D_0. Cependant, la régénération des membres chez des homards privés de leurs pédoncules oculaires semble inhibée.

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ACCORDING to accepted theory, bilateral eyestalk ablation will induce precocious ecdysis in decapod crustaceans (Passano 1960). Although there are published opinions that eyestalk ablation will accelerate molting in *Homarus americanus* (Donahue 1951, 1955) there are also opinions that it does not (Aiken 1969, Flint 1972). Only one paper (Flint 1972) presents data, but those can be questioned on the basis of factors known to affect the response of other decapods to eyestalk ablation (Sochasky 1973).[1]

Four independent eyestalk ablation experiments, conducted in this laboratory between 1967 and 1969, yielded inconsistent results. Operative procedures, holding conditions, and other experimental protocol differed, and postoperative mortality was usually high. This mortality masked the responses whether inhibition, acceleration, or no effect was indicated. Since factors such as sex, maturity, and molt cycle stage were not taken into account the question of the response of *H. americanus* remained unresolved.

In 1972, experiments were initiated to better describe the response of H. *americanus* to eyestalk ablation. Size, sex, maturity, molt cycle stage, limb regeneration, temperature, etc. — variables which may affect the response to eyestalk ablation (Sochasky 1973) — were taken into account. Shortly thereafter, Flint (1972) reported, "[Eyestalk ablation] in *Homarus* was not found to result in shortened intermolt...." Rather than no effect, Flint's data (1971, 1972) actually show a lengthened intermolt time since his eyestalkless lobsters molted *less often*. Although some of our earlier results could be interpreted this way, the experiments described here indicate that eyestalk ablation in *H. americanus*, as in other decapods, will cause precocious molting under certain conditions. Our examination of these conditions is not completed, but we are describing our initial results concerning molting and limb regeneration because we feel that the previous indications (Aiken 1969; Flint 1971, 1972) may not be representative of the response of this species.

Lobsters of about 60 mm carapace length (cl) were obtained in the fall from the Miminegash, Prince Edward Island, fishery. Males of the size range used possess "fully developed sperm" (Templeman and Tibbo 1945) and were probably mature. Females of this size range are seldom found carrying eggs in nature and those used in these experiments had abdominal widths characteristic of immature animals (Templeman 1944); however, all control females did assume an abdominal width characteristic of mature females at the first experimental ecdysis. Lobsters used previously, both here (unpublished data) and elsewhere (Flint 1971, 1972) were considerably larger (80–90 mm cl).

The molt cycle stage for each animal was determined at the start of an experiment and at intervals thereafter according to the classification system of Drach (1939) as modified specifically for *H. americanus* by Aiken (1973) involving setogenic changes in the pleopods (swimmerets). With this method, premolt curves can be constructed for each animal and data are obtained even when animals die before reaching ecdysis.

Intact animals were selected for these experiments insofar as possible but a few lacked one or two pereiopods (walking legs). Since limb regeneration has been used as a molt cycle staging technique (Bliss 1960, Flint 1971, 1972) the regeneration of these missing limbs which had all been autotomized before selection, was monitored by measuring limb bud growth[2], if any, when pleopod samples were taken.

In one experiment all eyestalks of 11 male and 15 female lobsters (60.2 $\pm$ 0.9 mm cl) were ablated with a polyethylene thread ligature. Premolt curves and times to ecdysis demonstrated that these eyestalk ablated lobsters completed proecdysis more rapidly than did comparable intact animals (from a different experiment; 6 male, 6 female; 51.3 $\pm$ 2.2 mm cl) held at the same temperature (Fig. 1). Times to ecdysis were 79 $\pm$ 5 and 105 $\pm$ 4 days, respectively ($P < .001$[3]).

On Day 0, six of the ablated lobsters were missing one leg and four were missing two legs. A total of nine limb bud papillas (basal growth) were present on seven of these lobsters on Day 0 but only three of these produced a regenerated limb at the first postoperative ecdysis. For the other five missing limbs on five animals there were no limb buds present on Day 0 and none developed before the first ecdysis. Another animal developed

[1]In a paper published after submission of this note Rao et al. (1973) have shown that eyestalk ablation accelerates molting in larval (fourth and fifth instar) *H. americanus*.

[2]Following autotomy a "limb bud" is typically formed at the autotomy plane. Depending on the stage of the molt cycle, the limb bud may grow and differentiate (premolt limb bud growth) within its protective sac or the limb bud may remain as a small papilla (basal limb bud growth plateau) (Bliss 1960). At ecdysis a "regenerated limb" is released only if premolt limb bud growth occurred. In *H. americanus* the papilla is usually less than 3 mm long during a basal plateau.

[3]All probabilities were determined using the Mann–Whitney *U* test (Siegel 1956). This test uses the data rather than calculated parameters, but average times to ecdysis and their standard errors are cited in the text for illustrative purposes.

a papilla for a pereiopod lost after Day 0 but it did not undergo premolt limb bud growth and therefore also failed to produce a regenerated limb.

In another experiment (Fig. 2) 20 lobsters (59.9 ± 0.6 mm cl) were divided into three treatment groups: A, bilateral eyestalk ablation (5 male, 5 female); B, unoperated controls (3 male, 2 female); C, bilateral carapace injury consisting of 1 cm^2 cuticle removed, underlying tissue exposed and cuticle replaced (2 male, 3 female).

Three lobsters in group A died before ecdysis and one of these — a female possibly undergoing ovarian development — remained in early D_0 for 112 days (#27 in Fig. 2). Proecdysis and time to ecdysis (64 ± 5 days) for the remaining seven was greatly accelerated over either of the control groups (times to ecdysis of 111 ± 11 and 133 ± 2 days, $P < .003$ and .001 for groups B and C, respectively) which were not significantly different from one another ($P > 0.05$). Three eyestalkless lobsters — all females — survived to molt a second time, with intermolt times of 144, 152, and 198 days. No control lobster molted twice in the 1-year duration of the experiment.

At the start of this experiment 10 animals lacked one limb and one other lacked two limbs, but no limb buds were present. All six limbs missing from five animals in the two control groups (B, C) were regenerated at the first ecdysis; in contrast, none of the six limbs initially missing, or others lost subsequently, in the eyestalk ablated group was regenerated at the first postoperative ecdysis, and only one eyestalkless lobster (#27) formed any limb buds. Of the three eyestalkless lobsters that molted a second time, the first two produced onlysmall limb buds but no regenerates whereas the third, which was notable for her protracted intermolt (198 days) including vitellogenesis and oosorption, developed a large limb bud for a cheliped lost after Day 0 and produced a regenerate at her second ecdysis.

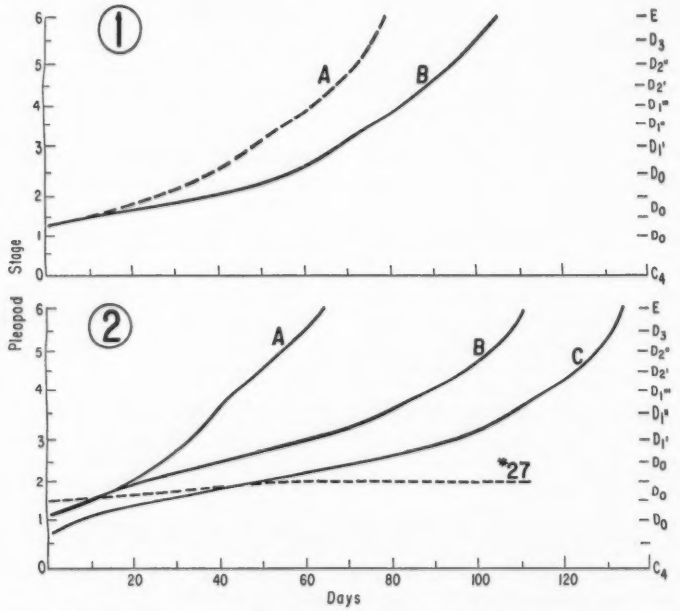

FIG. 1, 2. Effect of eyestalk ablation on premolt. (1) Averaged premolt curves for eyestalkless (A) and intact (B) lobsters. Day 0 was March 3, 1969 for intact and May 16, 1972 for eyestalkless animals. Both groups were held at seasonal seawater temperatures (< 6 C) prior to Day 0, and at 10 C thereafter; (2) Averaged premolt curves for eyestalkless (A), intact (B), and "bilateral carapace injury" (C) lobsters. One anomalous response from group A is plotted separately (# 27). Prior to Day 0 (February 24, 1972) they were held at the seasonal seawater temperature (0.1 C on Day 0) and at 10 C thereafter. Conventional molt stages (Aiken 1973) are given on the right margins.

Although limb bud growth curves may be suitable indicators of proecdysis in other decapods (Bliss 1960) and perhaps in normal lobsters, they are not at all satisfactory for eyestalkless lobsters. Both Flint (1971, 1972) and E. F. Couch (personal communication) have observed that eyestalkless *H. americanus* may not demonstrate limb regeneration prior to molting and our observations confirm this. Only three of 20 missing limbs were regenerated by the eyestalkless animals at their first postoperative ecdysis even though all limbs missing from the controls were regenerated. That some eyestalkless animals failed to regenerate missing limbs even though they had limb bud papillae at Day 0 or formed them after the ablations is similar to Démeusy's (1966) results on eyestalkless mature female *Carcinus maenas*. Eyestalk ablation in *H. americanus* may precipitate the "critical period" of the molt cycle (Tchernigovtzeff 1966), or eyestalk ablation may inhibit regeneration in some other way. The failure of some eyestalkless lobsters to regenerate limbs even by the second postoperative ecdysis may reflect some other complication. Since limb regeneration is obviously not an obligatory part of proecdysis, especially in eyestalkless lobsters, we conclude that to be universally applicable, a molt cycle staging technique must be based on phenomena, such as setogenesis, that are fundamental parts of the molt cycle.

Our results clearly indicate that *H. americanus* responds to bilateral eyestalk ablation with "accelerated molting" under the conditions of these experiments; that is to say, the response is consistent with that of other decapods. The principal "acceleration" seems to occur in early proecdysis (D_0), as the slopes of the individual curves for most lobsters between pleopod stages 3.0 and 6.0 (i.e. D_1' to ecdysis) appear essentially identical at 10 C. Time spent in D_0 in normal lobsters is variable because of a development plateau that is common in both immature and mature lobsters (Aiken 1973 and unpublished data), but D_0 seems consistently short in most eyestalkless lobsters. The principal effect of eyestalk ablation under our experimental conditions therefore seems to be in preventing or terminating the D_0 development plateau.

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Fatty Acids of Channel Catfish (*Ictalurus punctatus*): Variance Components Related to Diet, Replications Within Diets, and Variability Among Fish[1]

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WORTHINGTON, R. E., AND R. T. LOVELL. 1973. Fatty acids of channel catfish (*Ictalurus punctatus*): variance components related to diet, replications within diets, and variability among fish. J. Fish. Res. Board Can. 30: 1604–1608.

Channel catfish (*Ictalurus punctatus*) grown on six experimental diets were analyzed for fatty acid composition of total lipid. Estimates of components of variance showed that natural variability in fatty acid composition among fish due to genetic and/or other factors accounted for 5.5% of total variance. Variance associated with diet, replication, and error accounted for 92.8, 0.7, and 1.0% of total variance, respectively.

WORTHINGTON, R. E., AND R. T. LOVELL. 1973. Fatty acids of channel catfish (*Ictalurus punctatus*): variance components related to diet, replications within diets, and variability among fish. J. Fish. Res. Board Can. 30: 1604–1608.

Nous avons déterminé la composition en acides gras des lipides totaux de la barbue de rivière (*Ictalurus punctatus*) soumise à six régimes alimentaires expérimentaux différents. L'estimé des composantes de la variance démontre que les variations naturelles, parmi les poissons, de la composition en acides gras causées par des facteurs génétiques et/ou autres sont responsables de 5.5% de la variance totale. La variance associée au régime, à la réplique et à l'erreur est responsable de 92.8, 0.7 et 1.0% respectivement de la variance totale.

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PRODUCT composition is of considerable practical importance in commercial production of channel catfish (*Ictalurus punctatus*) as it may influence storage characteristics and consumer acceptance. Catfish grown under similar environmental conditions show a large degree of variability in length, weight, rate of gain, and body fat content. The extent to which this variability is genetically controlled has not been determined, however, several investigators have indicated a degree of genetic control over some of these characteristics in other fish species (Aulstad et al. 1972). Worthington et al. (1972) reported large variations in fatty acid composition of commercially processed pond-raised catfish, however, the source of this variability was not delimited but was assumed to be related primarily to differences in diet. The fatty acid composition of monogastric animals, including aquatic and marine species of fish (Castell et al. 1972 a,b; Stickney and Andrews 1971, 1972; Love 1970), is influenced by the fatty acid composition of the diet. The magnitude of the natural variability in fatty acid composition of catfish grown under controlled conditions, as compared to variability related to differences in dietary lipid, has not been reported.

The purpose of this report is to present results obtained from statistical analysis of fatty acid data from channel catfish grown from fingerling to harvestable size on six experimental diets containing various sources and amounts of animal and fish fat and in an environment with negligible amounts of natural food. Estimates of components of variance due to differences among diets, replication, and among fish are presented.

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Materials and methods — Six experimental diets (Table 1), differing primarily in type and level of fat content, were fed to channel catfish in eighteen 1/200-surface-acre open air concrete tanks at the Auburn University Fishery Research Unit, with each diet being replicated three times. All tanks were exposed equally to sunlight and atmospheric temperature. All fish were from the same spawn and were from the Auburn University strain of channel catfish into which no outside brood stock have been introduced in 10 years. Fifteen age-group 1 fish were stocked in each tank on July 20, 1971, and harvested 103 days later. The average initial weight was 26 g and the average weight of all fish at harvest was 328 g. The fish were fed once daily, 6 days per week, on the basis of 3% of biomass at the beginning of the trial with the allowance decreasing to 2% of biomass per day at the termination of the feeding period. Fish were sampled monthly for weight measurement, and feed allowance was adjusted every 2 weeks. During the course of the experiment the diets were stored at 5 C to impede oxidation of fatty acids. The ingredients and fatty acid compositions of the diets are shown in Table 1.

At harvest two fish were randomly selected from each tank and the head, viscera, and skin removed. The remaining portion of each fish was ground separately in a food chopper and thoroughly mixed. Total fat was determined by 4-hr diethyl ether extractions of freeze-dried samples. The remaining tissues were placed in small screw cap glass jars, frozen with dry ice, and maintained thereafter at –40 C. Subsequent analyses were made in the Food Science Laboratory of the Georgia Experiment Station.

At time of analysis, the glass jars were fractured and duplicate frozen samples (about 1 g) from each fish were freeze dried (an overnight operation) in a Virtis freeze dryer. Fatty acid methyl esters were prepared immediately after lyophilization, however subsequent investigations have shown that lyophilized tissue may be

TABLE 1. Composition of diets. Fatty acids listed as percent of total fatty acids, other ingredients as percent of air dry weight.

Ingredient	Diet 1	2	3	4	5	6
Catfish meal	15.0	15.0	15.0	15.0	15.0	.0
Herring meal	.0	.0	.0	.0	.0	11.4
Soybean meal	23.5	23.1	22.7	23.5	23.5	23.4
Peanut meal	23.6	23.0	22.7	23.6	23.6	23.4
Corn meal	28.2	32.8	37.0	28.2	28.2	29.6
Catfish oil	7.3	.0	3.5	.0	.0	.0
Menhaden oil	.0	.0	.0	7.3	.0	.0
Soybean oil	.0	.0	.0	.0	7.3	7.3
Vitamin mix[a]	.5	.5	.5	.5	.5	.5
Methionine	.2	.2	.2	.2	.2	.04
Carboxy methyl cellulose	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate	.0	.0	.0	.0	.0	2.5
Total fat	10.2	3.2	6.6	10.2	10.2	10.0
Total protein	32.0	32.0	32.0	32.0	32.0	32.0
Fatty acids[b]						
14:0	1.65	1.44	1.00	6.81	.53	1.96
16:0	18.41	16.73	17.18	18.87	14.69	12.97
16:1ω7	4.36	3.50	2.56	10.37	1.15	1.82
18:0	4.22	4.24	4.16	4.51	4.28	3.80
18:1ω9	43.56	39.95	35.73	21.68	38.26	36.22
18:2ω6	20.43	25.72	31.84	14.97	34.88	36.44
18:3ω3 + 20:1ω11	2.40	2.53	2.07	2.29	3.30	2.96
20:3ω9	.58	.57	.71	.30	.28	.29
20:4ω6	.63	.55	.47	.88	.26	.10
20:5ω3	.40	.60	.30	8.62	.35	1.22
22:5ω3	.23	.27	.15	1.40	Trace	.18
22:6ω3	.98	1.00	.63	4.10	.49	1.19

[a]Vitamin mixture (mg/kg diet): vitamin A acetate (325 USP units/mg), 17.0; *dl-a*-tocopherol acetate, 33.0; vitamin D_3 (200 ICU/mg), 22.0; vitamin B_{12}, 0.028; riboflavin, 16.5; choline chloride, 1.542.0; niacin, 99.1; pantothenic acid, 60.6; thiamine, 220.0; menadione, 4.4; pyridoxine, 5.5; folic acid, 1.1; biotin, 0.0441; ethoxyquin, 198.3.

[b]average of two determinations.

maintained at –40 C for up to 6 months without significant loss of unsaturated acids (unpublished data). In order to minimize oxidation of polyenoic acids, the extraction and transesterification of total lipid (neutral and phospholipid) were combined into a single operation (Worthington et al. 1972). In addition, total lipid from a limited number of samples was extracted by the method of Bligh and Dyer (1959) and separated into neutral and phospholipid (Carroll 1963) prior to transesterification. All methyl esters were maintained under a nitrogen atmosphere at –10 C and were analyzed by gas–liquid chromatography within 24 hr after preparation. Peak identifications were established by a comparison of relative retention times and equivalent chain lengths with published values (Ackman 1963a, b; Hofstetter et al. 1965) and with values obtained with standard fatty acid methyl esters. Quantitative measurements were made on 1.85 m × 4.0 mm inside diameter glass columns packed with 10% ECNSS-S on 70/80 mesh Chromosorb W (AW) (DMCS). Other details of the analytical procedures employed have been described previously (Worthington et al. 1972).

The fatty acid data were subjected to an analysis of variance (Anderson and Bancroft 1952) and components of variance were obtained from the appropriate mean squares to estimate variance due to diet, replication within diets, and among fish. The latter was accepted as natural variability arising from genetic and/or other sources. Means were separated by the Duncan's multiple range technique (Kramer 1957).

Results and discussion — Estimates of components of variance obtained from the appropriate mean squares are presented in Table 2. Although the fish utilized in this study exhibited the usual differences in body fat content, size, and other morphological characteristics, variance among fish in fatty acid distribution was small and accounted for only 5.5% of total variance (Table 2). The magnitude of this variability was small but statistically significant for all fatty acids except myristic (14:0) and eicosapentaenoic (20:5ω3) acids. Error variance accounted for 1.0% of total variance. A portion of variance among fish appeared to be related to differences in body fat content in that those fish highest in carcass fat generally contained slightly lower levels of polyenoic and stearic (18:0) acid and higher levels of palmitic (16:0), palmitoleic (16:1), and oleic (18:1) acid. These differences are a reflection of differences in relative proportions of fatty acids in neutral and phospholipids, the latter containing, in most cases, higher levels of polyenoic acids. In this study, differences in fatty acid composition of neutral and phospholipids were less pronounced in those fish receiving menhaden oil. Neutral and phospholipid values for 22:6ω3 were 3.37 and 4.85%, respectively in fish receiving menhaden oil (average values from two fish). In contrast, those fish receiving catfish oil contained 0.82 and 10.31% 22:6ω3 in the neutral and phospholipid fractions, respectively (average values from two fish). Phospholipids are generally considered to be functional in nature and are present in rather constant amounts in relation to lipid- and water-free tissue, regardless of levels of carcass fat. In the fish examined in this study, the ratio of phospholipids to lipid-free tissue, dry weight basis, varied between .040 and .053 (avg .047). Comparable values for neutral lipids varied widely, depending upon carcass fat content, and ranged between .263 and .815 among the four fish examined.

TABLE 2. Estimate of components of variance for fatty acids from channel catfish fed six experimental diets.

Fatty Acid	σ^2 Diets	σ^2 Replications	σ^2 Fish	σ^2 Error
14:0	2.326**[a]	.045*	.010NS	.097
16:0	4.154**	.159NS	.755**	.122
16:1ω7	5.037**	.020NS	.076**	.041
18:0	.045*	–.075NS	.199**	.108
18:1ω9	30.582**	.412NS	3.044**	.355
18:2ω6	40.715**	–.037NS	.737**	.069
18:3ω3 + 20:1ω11	.060**	.003NS	.068**	.015
20:3ω9	.075**	–.006NS	.017**	.009
20:4ω6	.036NS	.033NS	.100*	.018
20:5ω3	3.006**	.003NS	.024NS	.115
22:5ω3	.429**	.003NS	.006**	.004
22:6ω3	1.686**	.092NS	.122**	.040
Percent of total variance	92.8	.7	5.5	1.0

[a] *,** mean square significant at the 5% and 1% level, respectively; NS, not significant.

Variance among replications was small (0.7% of total variance) even though fish in some tanks experienced oxygen stress which in turn adversely affected food consumption and fish growth.

The considerable effect of diet on carcass total fatty acid distribution as demonstrated in this study, is in agreement with results obtained by numerous investigators working with many species of monogastric animals, including catfish (Stickney and Andrews 1971, 1972). In this study, diet accounted for 92.8% of total variance in carcass fatty acid composition (Table 2). The fatty acid data are summarized in Table 3. Of the major fatty acids, palmitic and stearic closely approximate the distribution present in dietary lipids, regardless of level of dietary fat, while palmitoleic and oleic generally equal or exceed the distribution present in dietary fat. In contrast, levels of linoleic acid (18:2) in carcass fat were in all instances much lower than in dietary fat. However, the level of this fatty acid is strongly influenced by diet as indicated by range in values in Table 3. Stickney and Andrews (1972) reported a low value, 5%, of linoleic acid in catfish grown on a fat-free diet. Those fish receiving menhaden oil accumulated significant quantities of 20:5ω3, 22:5ω3, and 22:6ω3 fatty acids (Table 3). Levels of the remaining fatty acids were rather uniform, probably reflecting a similar uniformity in dietary levels of these acids.

Although the production of oxidative off-flavors in fish is influenced by many factors (Stansby 1971), certain of the fatty acids present in catfish might be expected to contribute to off-flavors in iced or frozen stored products. Linoleic acid — and perhaps other fatty acids possessing the ω6,9 and ω6,9, etc. configuration — upon autoxidation yields *n*-hexanal, an aldehyde that has been shown to contribute significantly to the odor and flavor of oxidative rancidity (Swift et al. 1949; Labuza 1971). Eicosapentaenoic (20:5ω3), docosapentaenoic (22:5ω3), and docosahexaenoic (22:6ω3), as well as other polyenoic acids present in menhaden oil, are readily assimilated and deposited in the body fat of monogastric animals, including catfish. These, and other fatty acids possessing the ω3,6,9 or ω3,6,9, etc. configuration, oxidize to produce 2-*trans*,4-*cis*,7-*cis*-decatrienal, a compound that has recently been identified as contributing to the fishy odor and flavor of oxidized oil (Meijboom and Stroink 1972). The actual requirements of catfish for the ω3 and ω6 families of fatty acids should be determined and dietary intake adjusted accordingly to minimize accumulation of undesirable quantities of these acids in tissue lipids.

TABLE 3. Total fat and fatty acid values for channel catfish fed six experimental diets.

	Diet											
	1		2		3		4		5		6	
	%	±[a]	%	±	%	±	%	±	%	±	%	±
Total fat[b]	31.1	12.4	26.3	5.4	20.6	8.7	29.5	4.6	29.5	5.2	31.8	2.6
Fatty acids[c]												
14:0	1.41b[d]	.07	1.31b	.10	1.27b	.14	4.84a	.72	.74c	.05	.97c	.08
16:0	17.37c	.76	18.45b	1.69	18.85a	.76	18.22b	.36	14.57d	.60	14.05e	.98
16:1ω7	4.49c	.29	4.43c	.49	5.11b	.43	8.21a	.35	2.07d	.14	2.20d	.15
18:0	4.18b	.74	4.17b	.39	4.14b	.32	3.92b	.33	4.53a	.29	4.57a	.39
18:1ω9	46.80a	2.86	47.28a	1.15	46.67b	2.98	32.68e	1.21	43.03c	1.01	41.10d	.69
18:2ω6	13.42c	.67	13.60c	.54	12.71d	1.02	10.67e	.19	24.06b	1.04	25.49a	1.24
18:3ω3 + 20:1ω11	3.00c	.90	2.96c	.30	2.70d	.42	3.17b	.14	3.30a	.20	3.47a	.14
20:3ω9	1.00b	.19	1.07b	.08	1.06b	.15	.49c	.04	1.33a	.14	1.09b	.10
20:4ω6	1.37a	.50	1.19a	.24	1.61a	.68	1.18a	.12	1.13a	.14	.84a	.11
20:5ω3	.58b	.25	.43b	.01	.50b	.27	4.79a	.29	.51b	.50	.65b	.07
22:5ω3	.42c	.11	.31d	.09	.35d	.15	1.95a	.11	.23e	.04	.49b	.07
22:6ω3	1.91b	.56	1.55c	.42	1.93b	.85	4.80a	.18	1.15d	.18	1.68c	.12

[a]One standard deviation.
[b]Percent, dry weight basis.
[c]Percent of total fatty acids.
[d]Horizontal values followed by same letter are not significantly different at the 5% level.

The low level of natural variability among catfish in fatty acid composition found in this study demonstrates that genetic and other factors are of minor importance as compared to diet in determining fatty acid composition. As a practical consequence, a high degree of product uniformity in fatty acid composition may be obtained when catfish are grown on a diet that is fixed and uniform in lipid composition. These data further suggest that studies involving fatty acid metabolism may be successfully carried out with relatively small numbers of fish.

Acknowledgments — We appreciate the technical assistance of S. Nolan and analysis of data by J. C. Elrod.

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Recent Articles on Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating establishment. The number following each title refers to the establishment listed below.

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Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Department of the Environment
Fisheries and Marine Service
Biological Station
St. John's, Newfoundland

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Additional Publications

The Board also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at Board establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

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Coming Next Month !

Temperatures selected seasonally by four fishes from western Lake Erie
C. A. Barans and R. A. Tubb

The Beverton-Holt model of a commercial fishery: optimal dynamics
C. Clark, G. Edwards, and M. Friedlaender

Divergent growth in two lake whitefish (*Coregonus clupeaformis*)populations **B. F. Bidgood**

Changes in the population of lake whitefish (*Coregonus clupeaformis*) in Lake Winnipeg
E. B. Davidoff, R. W. Rybicki, and K. H. Doan

Microcultures of brown bullhead (*Ictalurus nebulosus*) cells: their use in quantitation of channel catfish (*Ictalurus punctatus*) **J. B. Gratzek, M. H. McGlamery, D. L. Dawe, and T. Scott**

atty acid content of coho salmon, *Oncorhynchus kisutch*—a statistical approach to changes produced by diet **I. J. Tinsley, H. M. Krueger, and J. B. Saddler**

Fish viruses: isolation and identification of infectious hematopoietic necrosis in eastern North America **K. Wolf, M. C. Quimby, L. L. Pettijohn, and M. L. Landolt**

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First records of the gymnoblastic hydroid, *Ichthyocodium sarcotretis*, on the copepod, *Sphyrion lumpi*, from redfish of the northwest Atlantic **W. Templeman**

Analysis of energy flow and production in an unexploited marine flatfish population
J. C. MacKinnon

Digestive enzyme secretion after dietary variation in the American lobster (*Homarus americanus*)
R. J. Hoyle

Cardiovascular-respiratory activity during recovery from anesthesia and surgery in brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*)
A. H. Houston, C. L. Czerwinski, and R. J. Woods

Notes

Endrin uptake and release by fingerling channel catfish (*Ictalurus punctatus*)
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Identification of fungal encrustation on the shell of the snow crab (*Chinonoecetes bairdi*)
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Inability to detect spores of *Clostridium botulinum* in fish protein concentrates (FPC)
A. H. W. Hauschild, H. Pivnick, and L. W. Regier

Karyotypes and erythrocyte sizes of some diploid and triploid fishes of the genus *Poeciliopsis*
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Occurrence of American eels, *Anguilla rostrata*, in waters overlying the eastern North American continental shelf **C. A. Wenner**

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K. A. Dormaar and S. Corey

The skate, *Raja richardsoni* Garrick 1961, assigned to *Bathyraja* **W. Templeman**

Occurrence of DDT residues in beluga whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T. **R. F. Addison and P. F. Brodie**

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Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required. Authors are encouraged to submit abstracts in both English and French; those submitted in one language only are translated by the *Journal*.

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and at least 1 mm high when the illustration is reduced to the appropriate size. Drawings should be grouped to facilitate comparisons. Originals must be submitted and should be less than 50 cm in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Photographs should be printed on glossy paper with a wide range in tone. They should be cropped, squared, and mounted on white cardboard, with no spaces between those arranged in groups.

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal Fisheries Research Board Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries*. L'auteur doit fournir un *résumé*. Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être éxécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

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Liste de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C). Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

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Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral *one*, and between the capital letter O and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal
Fisheries
Research
Board
Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).
Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts;* en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Food and Agriculture Organization of the United Nations

EIFAC Symposium on Methodology for the Survey, Monitoring, and Appraisal of Fishery Resources in Lakes and Large Rivers

The European Inland Fisheries Advisory Commission of FAO has arranged for a symposium on evaluation of fish stocks in large inland water bodies. The Symposium will be held from May 2 to 4, 1974, in Aviemore (31 miles from Inverness), United Kingdom, preceding the 8th session of the Commission, to be held from May 6 to 10.

Objectives of the Symposium are indicated by the proposed subjects to be discussed by panels of experts, as follows:

Panel 1 — Objectives and strategy of sampling

Panel 2 — Netting techniques

Panel 3 — Stupefying gear

Panel 4 — Electronic methods

Panel 5 — Weirs, fish passes, and traps

Panel 6 — Hooking gear

Panel 7 — Techniques for sampling larval and juvenile fish

Panel 8 — Interpretation of data

Detailed information for prospective delegates is available from the Secretariat, at Via delle terme di Caracalla, Rome.

Canadian Conference on Freshwater Fisheries Research

The twenty-seventh Annual Meeting of the CCFFR will be held in the Main Hall, Government Conference Center, Ottawa, January 7 and 8, 1974. The Fisheries Research Board of Canada, Department of the Environment, will again sponsor this meeting. The President is W. B. Scott, Royal Ontario Museum, Toronto, the Secretary is J. C. Stevenson, and the Treasurer is J. Camp, both of the Fisheries and Marine Service, Department of the Environment, Ottawa. Program Chairman is M. H. A. Keenleyside, Department of Zoology, University of Western Ontario, London.

The Canadian Society of Environmental Biologists and the Canadian Society of Zoologists, will also meet in Ottawa, January 9, 10, and 11, 1974.

CCFFR Program

Monday, January 7

MORNING SESSION — Recent changes in fish populations of the Great Lakes.

Convener: K. H. Loftus
Ontario Ministry of Natural Resources
Research Branch, Maple, Ont.

AFTERNOON SESSION — The use of thermal effluents in aquaculture.

Convener: G. Ian Pritchard
Fisheries and Marine Service
Department of the Environment
Ottawa, Ont.

EVENING — Smoker, sponsored by the Department of Biology, University of Ottawa.

Tuesday, January 8

MORNING SESSION — A new look at the environmental impact of hydro dams.

Convener: Ian E. Efford
University of British Columbia
Vancouver, B.C.

AFTERNOON SESSION — Contributed papers.

Convener: Peter A. Dill
St. Francis Xavier University
Antigonish, N.S.

Gross structure of the adductor muscles of some pelecypods **C. M. Morrison and P. H. Odense** 1583–1585

Postglacial colonization of the Matamek River, Quebec, by fishes
G. Power, G. F. Pope, and B. W. Coad 1586–1589

Use of a silver-sulfide electrode for standardizing aqueous sulfide solution in determining sulfide in water **J. Barica** 1589–1591

Vocalization in the ringed seal (*Phoca hispida*) **I. Stirling** 1592–1594

Apparatus for recording avoidance movements of fish **E. Scherer and S. Nowak** 1594–1596

Redescription of *Gaetanus intermedius* Campbell (Calanoida: Copepoda) from the type locality
T. Park 1597–1600

Does eyestalk ablation accelerate molting in the lobster *Homarus americanus*?
J. B. Sochasky, D. E. Aiken, and D. W. McLeese 1600–1603

Fatty acids of channel catfish (*Ictalurus punctatus*): variance components related to diet, replications within diets, and variability among fish **R. E. Worthington and R. T. Lovell** 1604–1608

Recent articles on investigations published in other periodicals 1609

Additional publications *1610*

Guide to Authors and Typists *i–iv*

Journal of the Fisheries Research Board of Canada

Volume 30, No. 10, October 1973 JFRBAK30(10)1409–1610(1973)

Experimental approaches to limnology — an overview **D. W. Schindler** 1409–1413

Eutrophication of lake 227 by addition of phosphate and nitrate: the second, third, and fourth years of enrichment 1970, 1971, and 1972 **D. W. Schindler, H. Kling, R. V. Schmidt, J. Prokopowich, V. E. Frost, R. A. Reid, and M. Capel** 1415–1440

A syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments **M. P. Stainton** 1441–1445

A numerical model for determining integral primary production and its application to Lake Michigan **E. J. Fee** 1447–1468

Modelling primary production in water bodies: a numerical approach that allows vertical inhomogeneities **E. J. Fee** 1469–1473

Gas-exchange rates in a small lake as determined by the radon method **S. Emerson, W. Broecker, and D. W. Schindler** 1475–1484

Distribution and uptake of artificially introduced radium-226 in a small lake **S. Emerson and R. Hesslein** 1485–1490

Vertical eddy diffusion studies in the thermocline of a small stratified lake **R. Hesslein and P. Quay** 1491–1500

Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in lake 227 **D. W. Schindler and E. J. Fee** 1501–1510

Production of epilithiphyton in two lakes of the Experimental Lakes Area, northwestern Ontario **D. W. Schindler, V. E. Frost, and R. V. Schmidt** 1511–1524

Movements of phosphorus between its biologically important forms in lake water **D. R. S. Lean** 1525–1536

Measurement of adenosine triphosphate (ATP) in two Precambrian Shield Lakes of northwestern Ontario **J. W. M. Rudd and R. D. Hamilton** 1537–1546

Heterotrophic utilization of sucrose in an artificially enriched lake **B. M. Thompson and R. D. Hamilton** 1547–1552

(Individual reprints of the above papers are not available)

Effects of cadmium and copper on the oxidation of lactate by rainbow trout (*Salmo gairdneri*) gills **E. Bilinski and R. E. E. Jonas** 1553–1558

Description and distribution of new specimens of the fish *Lipogenys gilli* from the western North Atlantic **W. Templeman** 1559–1564

Bioassay procedures to evaluate acute toxicity of neutralized bleached kraft pulp mill effluent to Pacific salmon **J. C. Davis and B. J. Mason** 1565–1573

Notes

Mercury uptake and ion distribution in gills of rainbow trout (*Salmo gairdneri*): tissue scans with an electron microprobe **K. R. Olson and P. O. Fromm** 1575–1578

First record of bacterial kidney disease in an apparently wild salmonid in British Columbia **T. P. T. Evelyn, G. E. Hoskins, and G. R. Bell** 1578–1580

Apparatus for simulating motion of animals through the geomagnetic fields **S. A. Rommel Jr** 1581–1582

(Continued inside back)

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